LOWER CYANIDE DETOXIFICATION RATES IN KONZO – A TROPICAL SPASTIC PARALYSIS LINKED TO CASSAVA CYANOGENIC POISONING

By

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ABSTRACT

Objective

To determine whether the odds of having konzo, a tropical spastic paraparesis associated with food (cassava) cyanogenic exposure, were associated with lower cyanide detoxification rates (CDR) and malnutrition.

Study Design And Setting

A case-control study was conducted in the rural district of Kahemba, Democratic Republic of Congo.

Methods

One hundred and twenty-two children (5 to 17 year-old) with konzo as per the WHO criteria for the disease were age and sex-matched with 87 presumably healthy controls. Exposure to cassava cyanogens was ascertained by measuring the concentrations of thiocyanate (SCN) in plasma (P-SCN) and urines (U-SCN). Children with Height-for-Age z-score < - 2 were classified as stunted. CDR was measured as rhodanese enzyme activity expressed in milliseconds required to detoxify cyanide and yield one µmole of SCN per mg of protein [ms/(µmol/mg protein)] or, alternatively, the amount of SCN in millimoles produced per minute in one ml of plasma [mmolSCN/(ml plasma/min)] during the cyanide detoxification process. Data were analyzed using conditional regression models with the significance level set at 0.05.

Results

The mean (SD) U-SCN concentration in children with konzo was 522.3 (354.3) μmol/l, significantly higher than the 384.6 (223.7) μmol/l concentration in those without konzo ($P < 0.05$). The disease was associated with stunting (OR: 5.8; 95% CI: 2.7 – 12.8; $p < 0.01$; N= 83 paired groups) and higher U-SCN concentration (OR: 1.1; 95% CI: 1 – 1.20 per 50-μmol increase in U-SCN; $p < 0.03$; N = 47 paired groups). CDR appeared slower in children with konzo [mean (SD) CDR: 427.1 (217.8) ms/(μmol/mg protein) or, equivalently, 11.5 (4.5) mmol SCN/(ml plasma/min)] compared to those without konzo [410.4 (175.9) ms/(μmol/mg protein) or 12.5 (5.4) mmol SCN/(ml plasma/min)]. After adjusting for stunting and U-SCN concentration, the odds of developing konzo was reduced by 63% (95% CI: 11 – 85%, $p = 0.03$; N = 41 paired groups) for each 5 mmol SCN/(ml plasma/min)-increase in the CDR.

Conclusion

Poor cyanide detoxification combined with higher U-SCN concentrations in children with konzo suggest that they may be experiencing a higher exposure to cassava cyanogens relative to those without konzo. Children with konzo appear to be at risk for recurrent toxic injuries. Prevention of the disease may require both a safer processing of cassava prior to human consumption and strategies to enhance the human detoxification process of cyanide in those relying on cyanogenic cassava as the main source of food. Nutritional rehabilitation must be key to the prevention of the cassava related neurological diseases.

Key words: cassava – cyanide detoxification – konzo – neurotoxicity – rhodanese

I. STATEMENT OF RESEARCH QUESTION

Food (cassava, *a.k.a* tapioca) cyanogenic poisoning is associated with neurodevelopmental deficits that include a distinct disease entity known as *konzo* in sub-Saharan Africa (1-6). Subjects affected by konzo show a visible spastic abnormality of gait while walking and/or running. Deficits in fine motor control and exaggerated deep tendon reflexes with or without ankle clonus are found with a prevalence of up to 20% in the general population of konzo-affected areas suggesting that subclinical forms of cassava neurotoxicity may exist (3, 7-9). A recent neuropsychological study revealed cognition deficits in children with konzo and, to a lesser extent, in those living under similar conditions but with no paralysis compared to children from neighboring villages not affected by the disease (10). Whether the observed motor and cognition deficits seen in the children from konzo-affected areas share the same mechanisms has yet to be determined (10-12).

To date, scientific evidence indicates that outbreaks of konzo are associated with chronic malnutrition and heavy dietary reliance on insufficiently processed bitter (cyanogenic) cassava as the main source of food (1, 2, 4, 6, 13-26). Epidemiological and toxicological studies suggest that neurological insults arise from the ingestion of cassava cyanogens, which break down in the human gut to form neurotoxic compounds such as cyanide and related metabolites (6). While most subjects from konzo-affected areas rely on the same cyanogenic cassava as the main source of food, only a fraction (i.e. up to 20% of the total population) presents with neuropsychological deficits. Of these, varying degrees of severity are observed suggesting that there may be individual

factors of susceptibility to cassava cyanogens and/or to their neurotoxic metabolites (6, 8, 9, 27). Current knowledge indicates that younger children and females are at higher risk for konzo. However, the biologic mechanisms underlying this susceptibility are not clearly understood (6). As for other types of toxicant-induced neuropathy, it is possible that poor detoxification of cyanide may contribute to the risk for cassava neurotoxicity (6, 28-30). In this study, we sought to determine whether poor cyanide detoxification rates, possibly driven by chronic malnutrition, are associated with konzo.

II. BACKGROUND AND REVIEW OF RELEVANT LITERATURE

II.1. The History of Konzo

Konzo (**Figure 1**) was first documented in 1938 in the southwestern region of Zaïre, the former "Belgian Congo", presently known as the Democratic Republic of Congo (DRC). However, the literature indicates that the disease was already known to the local populations of the Bandundu province in the DRC in the late 1800s (5). Konzo primarily affects children and women of childbearing age for reasons that have yet to be elucidated (31). The disease was named after a local designation in *kiyaka*, a DRC spoken language. The local designation, which means “tied legs” was made in reference to the scissoring and spastic gait of affected subjects (5, 32, 33).

II.2. Epidemiology and Neurological Impairments

To date, outbreaks of konzo have repeatedly occurred in many countries of sub-Saharan Africa including Mozambique (where it is called *mantakassa*)(34), Tanzania, Central African Republic, Cameroon, Angola, and Uganda. While isolated cases of the

disease may occur, konzo often occurs in an epidemic manner with prevalence rates of up to 5% in certain areas. The total number of cases is still underestimated due to the lack of reliable demographic censuses and data from surveillance systems. Several thousands of children and women of childbearing age have been affected and active outbreaks of the disease are still being reported (1, 2, 4, 18, 25, 35-38). The main clinical picture consists of a spastic paralysis of the legs. In severe cases, legs and arms are both affected and subjects may present with difficulties in speech and/or swallowing.

Figure 1. Severe form (child) and moderate form (woman with walking stick) of konzo in the Democratic Republic of Congo (Courtesy of Thorkild Tylleskär).

A recent neuropsychological study using the Kaufman Assessment Battery for Children, 2nd edition (KABC-II) for cognition and the Bruininks/Oseretsky Test, 2nd Edition (BOT-2) measure for motor proficiency showed that cognitive deficits may be part of the

neurological impairments associated with the disease (6, 10, 12). Current views on the disease picture suggest signs of cassava neurotoxicity may be better perceived on a continuum (gradient) model of neurological impairments with the possibility for the existence of pre-symptomatic konzo stages (12). Pre-symptomatic subjects may include those with minimal changes such as exaggerated deep tendon reflexes, ankle clonus, or subtle cognitive changes without signs of overt paralysis (8-10).

II.3. The Cassava Neurotoxicity Links

The biomarkers and mechanisms of konzo have been linked to the neurotoxicity of cassava for the last several decades. Epidemiological studies consistently show an association between the occurrence of konzo, chronic dietary reliance on insufficiently processed bitter (cyanogenic) cassava, and malnutrition (2, 4, 17, 35). Cassava (*Manihot esculenta* Crantz) is a drought-tolerant tropical shrub that is cultivated for its starchy storage roots and leafy vegetation (**Figure 2**) and is believed to be a staple for more than 600 million people in the tropics, half of whom live in Africa (39).

Figure 2. Harvested and unprocessed cassava roots (left) that are used to make cassava flour. Cassava leaves (right) are used as vegetables. Both contain cyanogenic compounds that breakdown in the human gut to produce cyanide.

Cassava cultivars are either “bitter” or “sweet”, the former generally contain higher amounts of cyanogenic glucosides, mainly linamarin and structurally similar lotaustralin (~ in a 93:7 concentration ratio) (40) (**Figure 3**).

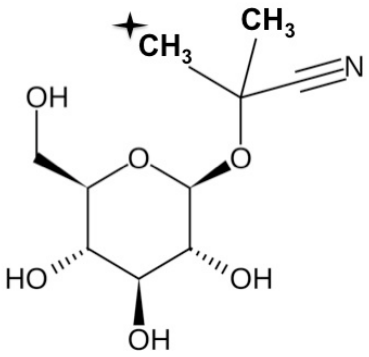

Figure 3. Chemical structure of linamarin, which is the main cassava cyanogenic compound. Its cyanogenic analogue, lotaustralin, instead has a CH_3-CH_2- (not a CH_3-) moiety linked to the side chain (star).

Cassava cyanogens must be removed prior to human consumption. Traditional processing methods to remove cyanogens and their degradation products include soaking raw cassava in water or grating the tuber followed by sun drying or heating and pounding. Upon disruption of the physical integrity of the cassava tubers, the β-glucosidases, which are mainly located in the cell walls, are liberated to degrade cyanogenic glucosides (14).

Improper processing of cassava may leads to cyanide poisoning and this is more likely to occur when adherence to effective processing techniques is no longer possible such as in times of famine caused by flood, drought, pestilence, or war (1, 17, 36, 41, 42). Under these conditions, larger amounts of cyanogenic compounds are ingested and

metabolized to cyanide (CN), a highly toxic compound. Under normal physiological conditions, cyanide is converted to the less toxic thiocyanate (SCN) *via* a rhodanese (thiosulfate sulfur transferase, TST)-mediated pathway. The efficacy of the TST-mediated detoxification pathway depends on the availability of sulfur donors (e.g. cystein and/or methionine), which provide sulfur for the conversion of CN into SCN. Protein catabolism may be enhanced to compensate for any deficiency in dietary intake of sulfur donors notably in the case of extreme limitations in food accessibility leading to chronic malnutrition (**Figure 4**).

Cyanide poisoning occurs after ingesting food products prepared from insufficiently processed cassava. Several studies have indicated that urinary concentration of thiocyanate (U-SCN) is a good marker of cassava cyanogenic poisoning. Plasma concentration of SCN (P-SCN) may reach a plateau and therefore may not be a good maker of cyanide poisoning (43).

Recent studies have shown that control of konzo is possible when interventions lower the mean concentration of U-SCN to less than 350 µmolSCN/l and this may be achieved by promoting safer processing of cassava prior to human consumption (13-16). Enhancing the cyanide detoxification process at the level of human metabolism, i.e. after individuals have been exposed, has not been tested, though it represents a promising approach to the prevention of the neurotoxic effects of cassava cyanogens.

Cassava tubers are detoxified following traditional processing methods (*vide supra*) and the detoxification process of cassava-derived food products may continue in the gastro-intestinal tract as illustrated in Figure 4.

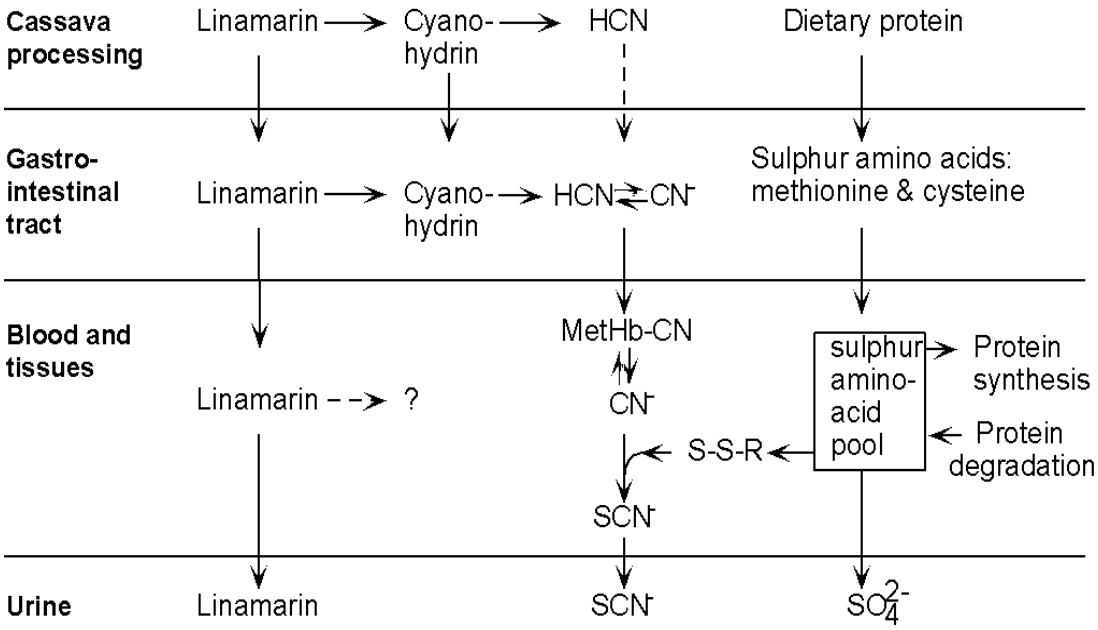

Figure 4. Fate of linamarin and cyanide during cassava processing and after ingestion of poorly processed cassava foodstuffs. Once the physical integrity of the cassava tissue is disrupted through soaking and/or pounding, linamarin is hydrolyzed to glucose and cyanohydrins. At pH > 5, the cyanohydrins spontaneously breakdown into ketones and hydrogen cyanide gas escapes. Lower pH leads to persistence of cyanohydrins in the finished food product, with the result that cyanide may be released by bacterial enzymatic cleavage in the lower gastrointestinal tract to enter bloodstream. Once in the bloodstream, cyanide is either trapped by methemoglobin (MetHB) or converted into thiocyanate (SCN). The human body may then excrete either intact linamarin or reportedly less toxic SCN in urine.

III. RESEARCH HYPOTHESIS

We hypothesized that susceptibility to cassava cyanogenic poisoning is associated with impaired cyanide detoxification, which is related to the nutritional status of individuals living in konzo-affected areas.

IV. SPECIFIC AIMS

To test our hypothesis, we addressed the following specific aims:

Study Aim 1. To determine whether the odds of having konzo are associated with lower cyanide detoxification rates, stunting, or the interaction of these two factors.

Study Aim 2. To determine whether quantitative measures of cognition or motor deficits in konzo using KABC-II or BOT-2 testing batteries, respectively, were associated with stunting, lower cyanide detoxification rates, or the interaction of the two factors.

V. METHODS

V.1. Study Implementation

We first carried out a survey in August 2012 to confirm an ongoing outbreak of konzo in Kahemba, a district with the highest prevalence of the disease in the DRC. For instance, over 3,000 children were reported to have konzo in 2011 alone (Reports DRC Ministry of Health). Interviews with village leaders revealed that the local population had endured food shortage on several occasions, notably in times of armed conflicts and displacement of the population during the Angola war that forced residents to rely

almost exclusively on bitter cassava as the main source of food (38). During the initial visit to Kahemba, samples of cassava flour from 18 consenting households were collected and analyzed for cyanide concentrations which were between 30 to 200 ppm, well-above the 10 ppm safe limit proposed by WHO (Joint FA0/OMS report on food contaminants, Rotterdam, 2009). In September 2012, we designed a case-control study to elucidate the factors associated with the Kahemba outbreak of konzo, which appeared to be the largest in the history of the disease (38).

V.2. Case-control Design

Children aged 5 - 17 years and suspected of having konzo were identified through the medical records from the Kahemba primary care clinic and interviews with key informants and church/radio announcements, as the disease was well known to villagers. To be formally included in the study, cases had to fulfill the following World Health Organization (WHO) criteria for the disease: (1) a visible symmetric spastic abnormality of gait while walking or running; (2) a history of onset of less than 1 week followed by a non-progressive course in a formerly healthy person; and (3) bilaterally exaggerated knee or ankle jerks without signs of disease of the spine. The severity of konzo was graded as follows: (1) mild (subject able to walk with no support); (2) moderate (subject needs support to walk), and (3) severe (subject unable to walk) (44). Presumably healthy control children as per the interview with a physician of the study team were recruited from the same population and tentatively age- and gender-matched to the cases (1:1-4 case-control ratio). Only those who were residents of Kahemba at the time of the outbreak and disease-onset were eligible to participate as control

subjects. Children with a past history of illness that may affect the nervous system (e.g., cerebral malaria, HIV I/II, or HTLV-I/II infections) were excluded.

Ethics statement

Informed consent and child assent were obtained verbally by investigators who were fluent in Lingala and/or Kikongo, the local spoken languages. Parents who allowed their children to participate in the study were then asked to sign a consent form that was kept on record at the study office. Ethical approval of research activities including informed consent and assent procedures was obtained from the Oregon Health & Science University (OHSU) Institutional Review Board FWA00000161 and from the Ministry of Health of the Democratic Republic of Congo (DRC).

V.3. Exposure and Outcome Measurements

Sample collection and storage

A team of trained laboratory technicians collected samples from people living in the remote and rural district of Kahemba in the DRC. Blood was collected through venipuncture in Vacutainer tubes with EDTA and kept at room temperature for approximately 2 hours. The blood was centrifuged at 15,000 rpm for 15 min, and the plasma was aliquoted in cryotubes and flash-frozen in liquid nitrogen. Samples were then shipped to Kinshasa, the capital city of DRC, and stored at – 80°C until shipment to OHSU (Portland, Oregon, USA) on dry ice for biochemical analyses. One-time spot urine collections were obtained at the time of the blood sample collection. Samples

were also immediately flash-frozen in liquid nitrogen, shipped to Kinshasa, and stored at – 80°C until use for biochemical analyses in Kinshasa.

Ascertainment of Cyanogenic Exposure

Exposure to cyanogenic compounds was ascertained by measuring U-SCN and P-SCN concentrations as previously reported (10, 12, 45).

Assessment of Nutritional Status

Height-for-age z-score calculated according the US National Center for Health Statistics (HAZNCHS) was used to assess the nutritional status of the study participants (http://www.cdc.gov/nchs/). Standing height was measured using a wall-mounted stadiometer, the subject stood erect on the floorboard of the stadiometer with his/her back to the vertical board. Gentle pressure was applied to the subject's knees and feet to obtain the correct position prior to recording the height to the nearest whole centimeter. HAZNCHS z-scores were computed using EpiInfo7 software (http://wwwn.cdc.gov/epiinfo/7/index.htm). Children with the HAZNCHS z-scores < - 2 were classified as stunted.

Enzyme Assay for Cyanide Detoxification

Cyanide detoxification rates were assessed in plasma using the rhodanese assay developed by Sörbo (46). Briefly, a solution of 200 mM potassium phosphate buffer (pH 8.6) was mixed with 125 mM sodium thiosulfate and 250 mM potassium cyanide. A 20 µl sample of plasma was added to the solution, mixed, and incubated at 37^{o}C for 20

minutes. The reaction was then stopped by addition of 37% formaldehyde. The end product of the reaction was SCN, which formed a red precipitate on mixing with an equal volume of 410 mM ferric nitrate solution. A standard curve was derived from a solution of 40 mM KSCN dissolved in deionized water and diluted accordingly. A 200 µl sample of the blank, each standard dilution and the plasma were assayed in duplicate. The absorbance of the final solution was read at or near 460 nm on a plate reader using the Epoch multivolume spectrophotometer system equipped with Biotek Gen 5 data analysis software (Biotek instruments, Inc, USA). The concentration of SCN in each sample was calculated from a standard curve of the KSCN solution (0.05 – 40 mM). The enzyme activity was expressed in µmole of SCN formed per minute per mg of protein at pH 8.6 and 37^{o}C. This was later expressed as the number of milliseconds required to detoxify cyanide to yield one µmol of SCN per mg of protein [ms/(µmol/mg protein)] in the tested sample or, using an alternative unit i.e. the number of millimoles of SCN produced per min in one ml of plasma [mmolSCN/(ml plasma/min)] after taking into consideration the individual concentrations of proteins in plasma.

Neurological Outcomes

Each child's disease status was primarily determined as "konzo" or "non-konzo" according to the WHO criteria for the disease. The same criteria were used for the severity of the disease. Each child was then assessed using the BOT-2 and KABC-II for motor and cognitive performance, respectively. BOT-2 is regarded as one of the most comprehensive and sensitive instruments for motor assessment. Testing involves game-like tasks that hold the subject's interest and are not verbally complex. The BOT-

2 has proven adaptable and useful in characterizing the specific aspects of motor impairment associated with HIV in Ugandan children, a disease which also has pediatric neuromotor effects (47). The KABC battery has been validated in Uganda and used in the DRC prior to this study (47-49). Its global mental processing index, herein referred to as KABC-II score, was used as the quantitative measure of cognition in this study. Similarly, the BOT-2 total composite score was used as a measure of motor proficiency.

V.4. Statistical Analysis

The disease status (konzo vs. non-konzo) or the quantitative BOT-2 and KABC-II test scores were used as main outcomes. Cyanide detoxification rates (CDR), P-SCN and U-SCN, and nutritional status (HAZNCHS z-scores or stunting status Yes/No) were the main predictors. Initial analyses used Student *t* test, ANOVA, Mann-Whitney, or Kruskal-Wallis tests to compare key characteristics as related to exposure to cyanogenic compounds, nutritional status, CDR as well as neurocognitive performance. Conditional logistic regression determined the odds of having konzo in relation to the aforementioned predictors. Linear regression determined whether the quantitative measures of neurocognitive performance, i.e. BOT-2 and/or KABC-II scores, were associated with the same predictors listed above. Standard checks for model adequacy (Shapiro-Wilk test and Q-Q plot of residuals, plots of residuals against fitted values, screening for outliers/high leverage) were performed for each of the applicable models. The STATA software (version 11.2) was used for all analyses with the significance level set at $p \leq 0.05$.

VI. STUDY FINDINGS

VI.1. Age and Sex Distribution

A total of 209 subjects was included in the study, of whom 117 (aged 4.6 – 17.6 years) were males and 92 (aged 4.3 – 16.3 years) were females. Of the 209 children, 122 [aged 4.3 – 17.6 years; mean (SD): 8.7 (2.6)] were affected by konzo while 87 [aged 4.7 – 15.4 years; mean (SD): 9.1 (2.6)] served as controls. Among those affected by konzo, 65 (53.3%) were boys and 57 (46.7%) were girls whereas 52 (59.8%) and 35 (40.2%) were boys and girls, respectively, among the controls.

VI.2. Motor and Cognition Deficits

Of the 122 children with konzo, 91 (74.6%) had a mild form of the disease, whereas 18 (14.7%) and 13 (10.7%) had either a severe or a moderate form of konzo, respectively. The duration of the disease since onset of konzo ranged from 1 to 101 months with a mean (SD) duration of 27.1 (21.2) months. The median (IQR) BOT-2 scores for motor proficiency were 22 (20 – 29) in children with konzo relative to 34 (31 – 41) in those not affected by the disease ($p < 0.01$, Mann-Whitney test). The median (IQR) KABC-II scores for cognition were 58 (52 – 65) in children with konzo relative to 60 (54 – 68) in those not affected by the disease ($p = 0.03$, Mann-Whitney test). Severely affected subjects performed worse on the BOT-2 testing of motor proficiency.

Both the BOT-2 and KABC-II median scores changed in accordance with the severity of the disease ($p < 0.01$, Kruskal-Wallis test) (**Table 1**).

Table 1. Motor (BOT-2) and cognition (KABC-II) performance scores by disease status and severity.

Performance Scores	Non Konzo (N = 87)	Mild Konzo (N = 91)	Moderate Konzo (N = 13)	Severe Konzo (N= 18)
BOT-2 Scores				
Mean (SD)	35.3 (7.6)	26.0 (6.4)	22.0 (2.4)	20.9 (2.3)
Median (IQR)	34 (31 – 41)	24 (20 – 31)	21 (20 – 24)	20 (20 – 20)
KABC-II Scores				
Mean (SD)	61.4 (9.1)	59.4 (7.7)	60.9 (9.5)	52.6 (7.2)
Median (IQR)	60 (54 - 68)	59 (52 - 65)	62 (54 – 66)	52 (45 – 56)

Both BOT-2 and KABC-II median scores changed with respect to the disease status and severity of the disease. Severely affected children tend to perform poorly relative to their counterparts except for moderately affected children who scored higher at the KABC-II testing (p < 0.01, Kruskall-Wallis test).

VI.3. Nutritional Status in Relation to Konzo

The HAZNCHS z-scores were significantly lower in children with konzo [mean (SD): -3.4 (1.4)] relative to those without konzo [mean (SD): - 2.3 (1.8)] (P < 0.05, Student *t* test). No effect of sex was seen on these scores irrespective of konzo status (**Figure 5**). In the konzo group, 106 (86.89%) children were stunted (HAZNCHS z-score < -2)

compared to 47 (54.02%) in the non-konzo group ($p < 0.01$, Chi-square test). Almost all the children (17/18) severely affected by the disease were stunted.

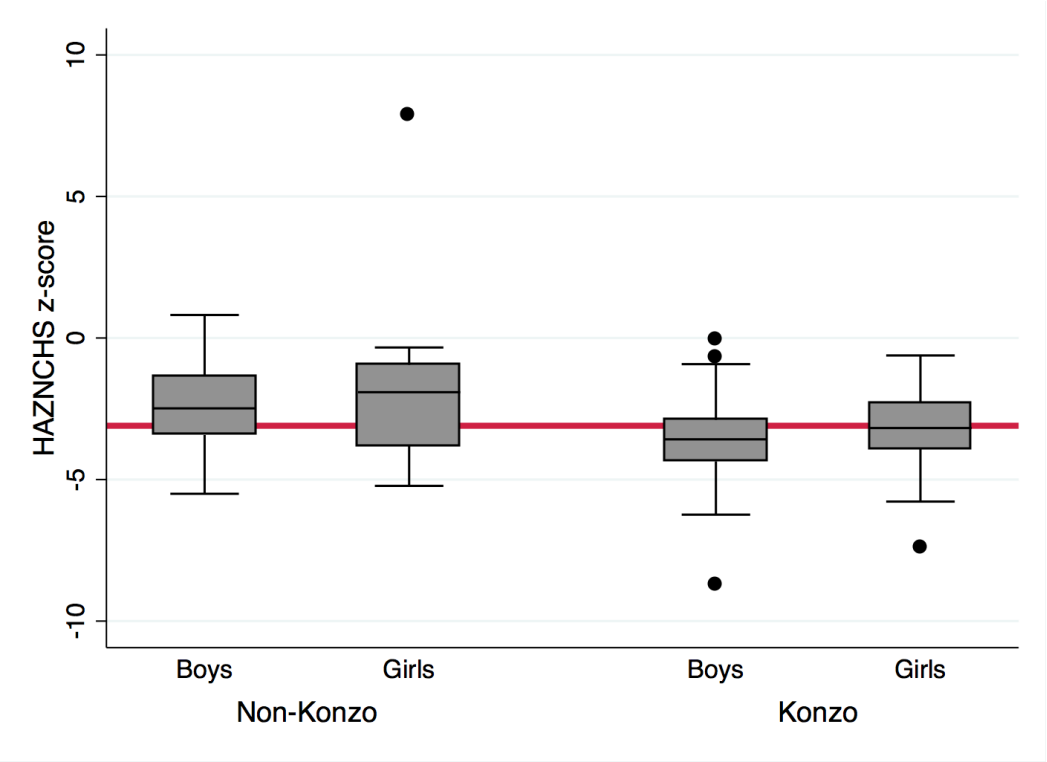

Figure 5. Children affected by konzo had lower median HAZNCHS z-scores compared to those not affected by the disease. No significant difference was seen between boys and girls irrespective of disease status. The median HAZNCHS z-score of children with konzo was lower or equal to the overall median score of – 3.1 (Y-axis reference line).

VI.4. Cyanogenic Exposure and Cyanide Detoxification Rates

Overall, the concentrations of U-SCN and P-SCN ranged from 17.2 to 1720 µmolSCN/l and 64 to 426 µmolSCN/l, respectively. The mean (SD) and median (IQR) P-SCN were 228.1 (69.8) and 224.5 (179.0 – 279.4) µmol/l, respectively. For U-SCN, the overall mean (SD) and median (IQR) were 474.5 (321.1) and 344 (144 – 688) µmol/l, respectively. U-SCN positively correlated with P-SCN (Spearman $r = 0.28$, $p = 0.01$)

(**Figure 6**). With respect to the disease status, children with konzo had U-SCN and P-SCN ranging from 17.2 to 1720 µmol SCN/l and 64 to 426 µmol SCN/l, respectively. Those without konzo had U-SCN and P-SCN that ranged from 34.4 to 1032 µmol SCN/l and 69 to 410 µmol SCN/l, respectively.

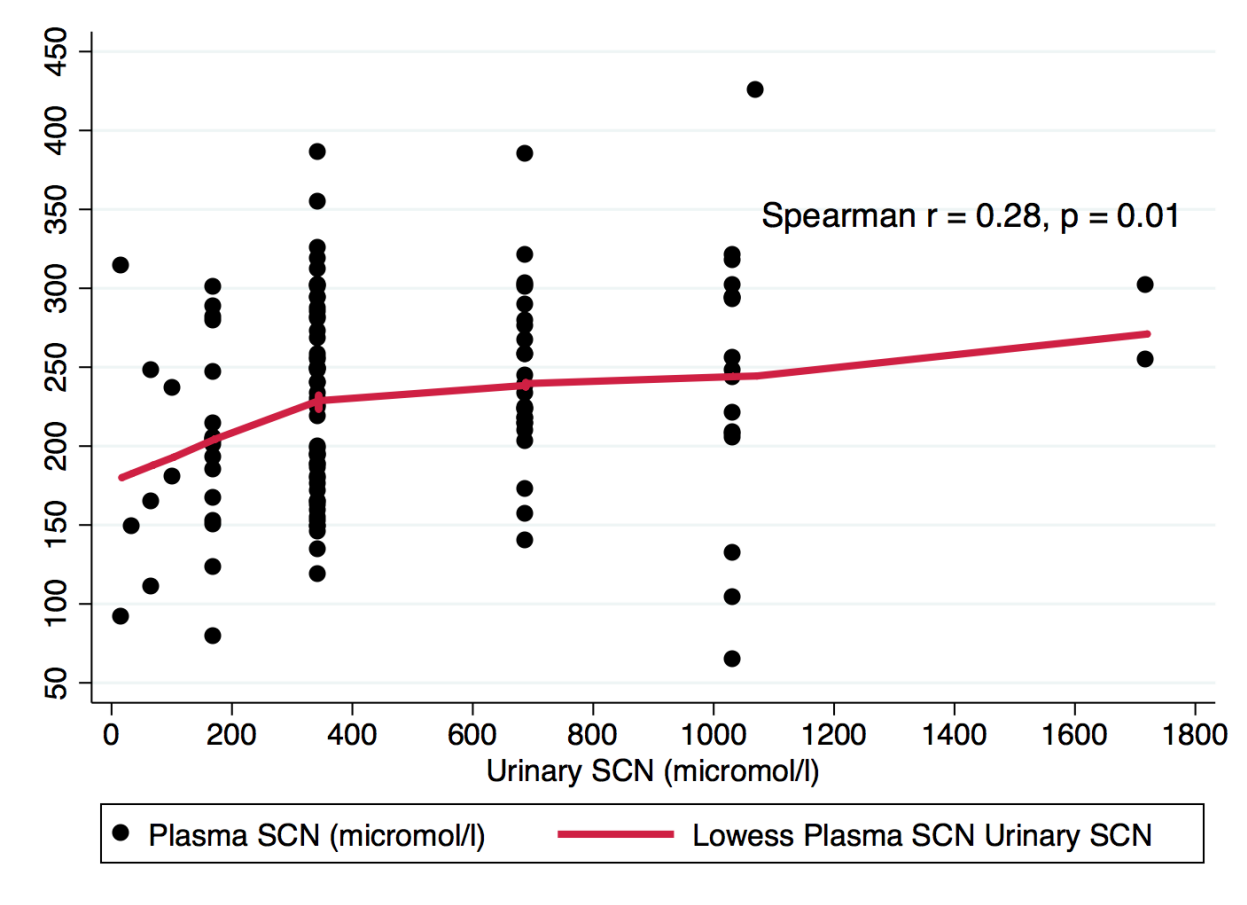

Figure 6. Children with higher concentration of plasma SCN tend to excrete more SCN in urine. However, the figure also indicates that higher plasma SCN may also be accompanied by poor urinary excretion of SCN or vice-versa.

However, with respect to the nutritional status, the concentrations of U-SCN ranged from 17.2 to 1720 µmol SCN/l in the stunted group relative to 172 to 1072 µmol SCN/l in

the non-stunted group. P-SCN ranged from 64 to 426 µmol SCN/l in the stunted group relative to 117 to 425 µmol SCN/l in the non-stunted group.

Overall, CDR ranged from 78.9 to 1670 msec/(µmolSCN/mg protein) or, equivalently, from 2.5 to 30.1 mmolSCN/(ml plasma/min). The mean (SD) CDR was 420.1 (201) msec/(µmolSCN/mg protein) or, equivalently, 12.0 (4.9) mmolSCN/(ml plasma/min). The median (IQR) was 383.6 (278.1 – 519.9) msec/(µmolSCN/mg protein) or, equivalently, 11.1 (8.3 – 14.5) mmolSCN/(ml plasma/min). With respect to the nutritional status, stunted children had CDR that ranged from 78.9 to 1670 msec/(µmolSCN/mg protein) or 2.5 to 30.1 mmolSCN/(ml plasma/min) compared to 134.1 to 1053 msec/(µmolSCN/mg protein) or 4.7 to 26.3 mmolSCN/(ml plasma/min) in those apparently well nourished. Children with konzo had CDR ranging from 78.9 to 1670 msec/(µmolSCN/mg protein) or 2.5 to 26.3 mmol SCN/(ml plasma/min) compared to 104.8 to 1053 msec/(µmolSCN/mg protein) or 4.3 to 30.1 mmol SCN/(ml plasma/min) in those without konzo. No significant correlation was found between CDR and U-SCN or P-SCN irrespective of the nutritional or disease status. The mean (SD) and median (IQR) CDR, U-SCN and P-SCN in relation to stunting and disease status are respectively summarized in **Table 2, Table 3a, and Table 3b**.

Table 2. Cyanide detoxification rates (CDR) and plasma and urinary thiocyanate (SCN) concentrations by stunting.

Variables (units)	**Stunting**	**No Stunting**
CDR [mmol SCN/(ml plasma/min)]	N = 148	N = 51
Mean (SD)	12.1 (5.1)	11.7 (4.4)
Median (IQR)	11.1 (8.3 – 15.0)	11.7 (8.7 – 14.0)
CDR [msec/(µmolSCN/mg protein]		
Mean (SD)	409.8 (205.3)	445.4 (188.4)
Median (IQR)	382.7 (264.0 – 513.8)	382.1 (308.4 – 592.5)
Plasma SCN (µmol/l)	N = 133	N = 47
Mean (SD)	225.5 (70.4)	235.9 (69.3)
Median (IQR)	225 (176 – 281)	224 (198 – 279)
Urinary SCN (µmol/l)	N = 108	N = 34
Mean (SD)	474.6 (333.4)	471.6 (290.6)
Median (IQR)	344 (344 – 688)	344 (344 – 688)

No differences were seen between mean or median CDR, P-SCN, or U-SCN of children who were stunted relative to those who were not stunted ($p > 0.05$; Student *t*-test or Mann-Whitney test for mean or median comparisons, respectively).

Table 3a. Cyanide detoxification rates (CDR) and plasma and urinary thiocyanate (SCN) concentrations by disease status.

Variables (units)	**Konzo**	**Non-Konzo**
CDR [mmol SCN/(ml plasma/min)]	N = 117	N = 84
Mean (SD)	11.5 (4.5)	12.5 (5.4)
Median (IQR)	10.8 (8.2 – 14.2)	11.6 (8.5 – 15.9)
CDR [msec/(µmolSCN/mg protein]		
Mean (SD)	427.1 (217.8)	410.4 (175.9)
Median (IQR)	388.0 (278.1 – 533.2)	382.8 (276.7 – 496.3)
Plasma SCN (µmol/l)	N = 108	N = 74
Mean (SD)	230.9 (74.0)	224.0 (63.4)
Median (IQR)	226.5 (179.5 – 288.0)	224 (179 – 265)
Urinary SCN (µmol/l)	N = 94	N = 50
Mean (SD)	522.3 (354.3)	384.6 (223.7)
Median (IQR)	344 (344 – 688)	344 (344 – 344)

No differences were seen between mean or median CDR or plasma SCN among children with konzo relative to those without konzo (p > 0.05; Student *t*-test or Mann-Whitney test for mean or median comparisons, respectively). Mean urinary SCN in the konzo group was significantly higher than the mean concentration of the non-konzo group (p = 0.01, Student *t*-test).

Table 3b. Cyanide detoxification rates (CDR) and plasma and urinary thiocyanate (SCN) concentrations by disease severity.

Variables (units)	Mild Konzo	Moderate Konzo	Severe Konzo
CDR [mmol SCN/(ml plasma/min)]	N = 88	N = 13	N = 16
Mean (SD)	11.8 (4.4)	12.3 (5.6)	9.6 (3.6)
Median (IQR)	11.1 (8.4 – 14.4)	12.7 (7.1 – 13.7)	9.3 (7.6 – 11.6)
CDR [msec/(µmolSCN/mg protein]			
Mean (SD)	408.6 (191.0)	413.8 (187.2)	539.6 (333.9)
Median (IQR)	364.7 (269.5 – 513.8)	340.6 (261.8 – 592.5)	418.4 (390.2 – 592.1)
Plasma SCN (µmol/l)	N = 83	N = 12	N = 13
Mean (SD)	238.7 (73.1)	235.1 (74.3)	177.8 (61.4)*
Median (IQR)	239 (185 – 290)	219.5 (206.5 – 275)	162 (150 – 199)
Urinary SCN (µmol/l)	N = 68	N = 9	N = 17
Mean (SD)	501.7 (341.2)	879.1 (388.9)**	415.8 (284.1)
Median (IQR)	344 (344 – 688)	688 (688 – 1032)	344 (344 – 344)

***Mean plasma SCN concentration was lower in children with severe konzo relative to those with mild konzo (p = 0.02, one-way ANOVA, Bonferroni correction).**Mean urinary SCN concentration was higher in children with moderate konzo relative to those with mild konzo or severe konzo (*p* < 0.01 for all comparisons, one-way ANOVA, Bonferroni correction).**

VI.5. Neurological Deficits in Relation to Exposure, Nutritional Status, and Cyanide Detoxification Rates

Conditional logistic regression analyses showed that the odds of having konzo were associated with stunting, the concentration of U-SCN, and CDR as measured by the amount of SCN produced per min per ml of plasma after adjusting for U-SCN (**Table 4**). The analysis that was conducted on 91 subjects (41 paired groups) with a complete set of data for the above mentioned predictors revealed that the odds of having konzo were reduced by 63 % (OR: 0.37; 95%CI: 0.15 – 0.89, p = 0.03) for each 5-mmol SCN/(ml plasma/min) increase (arbitrary choice) in the detoxification rate of cyanide. However, the odds of the disease were increased by 185 % (OR: 2.85; 95%CI: 1.0 – 8.3, p = 0.05) with stunting and by 20 % (OR: 1.2; 95%CI: 1.05 – 1.37, p = 0.01) for each 50-µmol increase (arbitrary choice) in the concentration of U-SCN in the same association model (**Table 5**).

Table 4. Odds of konzo in relation to cyanide detoxification rates (CDR) and plasma and urinary thiocyanate (SCN) concentrations.

Predictors (Units)	Unadjusted Odds Ratio (95% CI); p-value (N Paired Groups)	Adjusted for Stunting (95% CI); p-value (N Paired Groups)	Adjusted for Urinary SCN (95% CI); p-value (N Paired Groups)
CDR [mmol SCN/(ml plasma/min)]/5-unit change	0.75 (0.51 – 1.1) p = 0.15	0.67 (0.43 – 1.04) p = 0.08	0.41 (0.19 – 0.90) p = 0.03
CDR [msec/(µmol SCN/mg protein)]/100-unit change	1.06 (0.91 – 1.26) p = 0.43 (N = 78)	1.16 (0.96 – 1.40) p = 0.13 (N = 76)	1.20 (0.91 – 1.55) p = 0.20 (N = 43)
Stunting (Yes/No)	5.85 (2.68 – 12.79) p < 0.01	N/A	3.6 (1.4 – 9.6) p = 0.01
HAZNCHS z-score	0.54 (0.42 – 0.71) p < 0.01 (N = 83)	N/A	0.61 (0.43-0.86) p < 0.01 (N = 45)
Plasma SCN (µmol/l)/50-unit change	1.07 (0.86 – 1.32) p = 0.52 (N = 67)	1.25 (0.97 – 1.62) p = 0.08 (N = 65)	0.86 (0.60 – 1.25) p = 0.45 (N = 34)
Urinary SCN (µmol SCN)/50-unit change	1.10 (1.01 – 1.20) p = 0.02 (N = 47)	1.14 (1.02 – 1.26) p = 0.02 (N = 45)	N/A

Table 5. Odds of konzo in relation to cyanide detoxification rates (CDR) and plasma and urinary thiocyanate (SCN) concentrations in 41 matched pairs with a complete set of measurements.

Predictors (Units)	**Unadjusted Odds Ratio (95% CI) p-value**	**Adjusted for Stunting (95% CI) p-value**	**Adjusted for Urinary SCN (95% CI) p-value**	**Full Model (95% CI) p-value**
CDR [mmol SCN/(min/ml plasma]/5-unit change	0.58 (0.31 – 1.1) p = 0.09	0.51 (0.25 – 1.02) p = 0.06	0.37 (0.16 – 0.86) p = 0.02	0.37 (0.15 – 0.89) p = 0.03
Stunting (Yes/No)	2.35 (0.96 – 5.82) p = 0.06	N/A	2.71 (1.0 – 7.36) p = 0.05	2.85 (1.0 – 8.3) p = 0.05
HAZNCHS z-score	0.69 (0.49 – 0.97) p = 0.03	N/A	0.69 (0.48-0.98) p = 0.04	0.65 (0.48-0.98) p = 0.03
Urinary SCN (µmol SCN)/50-unit change	1.14 (1.03 – 1.27) p = 0.01	1.16 (1.03 – 1.30) p = 0.01	N/A	1.2 (1.05 – 1.37) p = 0.01

Linear regression analysis suggested a significant association between the BOT-2 or KABC-II scores and both the HAZNCHS z-scores and the concentration of U-SCN but not the CDR. In non-parametric analyses, BOT-2 scores positively correlated with the HAZNCHS z-scores (Spearman $r = 0.44$, $p < 0.01$, $N = 90$) in the konzo group but not in the non-konzo group (Spearman $r = 0.25$, $p > 0.05$, $N = 48$). A similar trend was seen for the KABC-II scores, which positively correlated with the HAZNCHS z-scores (Spearman $r = 0.32$, $p < 0.01$, $N = 90$) in the konzo group but not in the non-konzo group (Spearman $r = 0.22$, $p > 0.05$, $N = 48$). Both the BOT-2 and KABC-II scores, however, failed to correlate with CDR.

VII. DISCUSSION

We report for the first time plasma cyanide detoxification rates in children from cassava areas that are affected by konzo. Children with konzo require up to 1670 msec to produce one µmol of SCN/mg protein during the detoxification process of cyanide in their plasma, which corresponds to a detoxification rate ~ 3-fold slower than the average observed in non-human primates *macaca fascicularis* or a rate ~ equivalent to 1.5-fold the rates observed in rodents. Detoxification rates in children without konzo were up to 1050 msec/µmolSCN/mg protein, which are equivalent to the rates reported for rodents (50). Human rates close to those seen in small mammals notably rodents suggest that even children without konzo may be at risk for cassava neurotoxicity. These findings may possibly explain poor performances at the neuropsychological testing of presumably health children from konzo areas as indicated by our previous findings (10).

Despite the lack of statistical significance in our study findings, stunted children may detoxify cyanide faster than those who are not stunted. This proposal would be consistent with previous studies that suggested that protein catabolism is enhanced in subjects on the cassava cyanogenic diet to provide sulfur for the detoxification of cyanide, which in turn may lead to stunting (31, 51). An additional consequence to this hypothesis would be that subjects on a cyanogenic diet may suffer from further neurological deficits due to changes in the metabolism of sulfur, which may be directed toward the detoxification process of cyanide at the expense of normal metabolic processes (12, 31).

After adjusting for stunting and urinary concentrations of SCN, we found that the odds of having konzo was reduced by 63% for each 5-unit increase in cyanide detoxification rates expressed as the amount of mmol of SCN produced per ml of plasma during a one-minute detoxification process of cyanide. This implies that the risk for konzo decreases as the cyanide detoxification capabilities increase. This finding appears to be consistent with changes in CDR by disease status and severity, which indicate that severely affected children tend to have poorer detoxification rates. Reverse causation, however, remains possible since the severely affected children may also disproportionably suffer from malnutrition and therefore, present with poor detoxification capabilities (6). The association between konzo and CDR in mmol SCN/(ml plasma/min), which were calculated based on the individual protein concentrations in plasma but not with CDR expressed in msec/(μmol SCN/mg protein) indicates that the

concentration of proteins in plasma may be key to the mechanisms underlying the individual susceptibility to konzo.

We failed to demonstrate a linear correlation between CDR and neurological deficits quantitatively measured using the BOT-2 and KABC-II testing batteries. Although a non-linear relationship may exist, this finding underlies the complexity and challenges encountered in the process of anchoring clinical phenotypes of chronic conditions to biomarkers whether of exposure, disease process, or intermediate metabolic changes. Impaired cyanide detoxification may indeed arise from several factors including genetic polymorphisms, cyanide toxicity itself, posttranslational modifications of proteins including oxidation and/or cyanate-induced carbamoylation, and deficiency in sulfur donors (6, 12, 28, 29, 50-52).

The limitations of our study include a small sample size of subjects who were not randomly selected to allow generalization of the study findings. This study has not explored non-enzymatic functions of cyanide detoxification. In addition, the case-control design does not allow us to rule out reverse causation. Nevertheless, we have shown that children with konzo are at risk for recurrent toxic injury due to poor cyanide detoxification capabilities. The combination of poor cyanide detoxification and higher U-SCN in children with konzo suggest that they may be enduring a higher exposure to cassava cyanogens compared to those presumably healthy. Prevention of the effects of cassava neurotoxicity including konzo may require both a reduction in the exposure

levels through a safer processing of cassava prior to human consumption and strategies to enhance the detoxification of cyanide in humans.

FUTURE DIRECTIONS

Our study findings support a theoretical framework that highlights recurrent risks for cassava neurotoxicity in subjects affected by konzo (**Figure 7**).

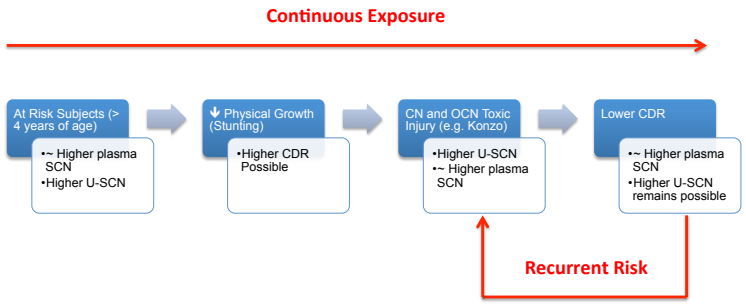

Figure 7. Theoretical framework of chronic cassava neurotoxicity. Children consuming cassava meals (i.e., those who are not breastfed) are at risk for cyanogens exposure and stunted growth due to protein catabolism to detoxify cyanide. Once cyanide detoxification capabilities are overwhelmed, subjects may develop signs of toxicity including konzo. Continuous exposure to cyanide and metabolic changes induced by cyanide and/or cyanate, or nutritional deficiencies, may further impair individual cyanide detoxification capabilities putting those already neurologically impaired at risk for further neurotoxicity.

Longitudinal follow-up studies should allow us to measure risks for recurrent toxic injury in subjects with konzo or any possible subclinical signs associated with cassava toxicity. Experimental modeling of cyanide toxicity including strategies to enhance the detoxification of cyanide may open new lines of public health interventions to prevent cassava associated neurological diseases. Whether lower detoxification rates in subjects with konzo are mediated through genetics, deficiencies in select nutrients, or metabolic changes induced by cyanide or related metabolites such as the carbamoylating agent cyanate has to be determined. The potential for multiple and recurrent toxic injuries in subjects living in konzo areas warrants studies to assess the global burden of disease associated with chronic dietary reliance on cassava as the main source of food.

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