Published in final edited form as:

Neurotoxicology. 2017 March ; 59: 256–262. doi:10.1016/j.neuro.2016.05.016.

Lower sulfurtransferase detoxification rates of cyanide in konzo —A tropical spastic paralysis linked to cassava cyanogenic poisoning

K.J. Kambale^a, E.R. Ali^{b,k}, N.H. Sadiki^c, K.P. Kayembe^c, L.G. Mvumbi^a, D.L. Yandju^b, M.J. Boivin^d, G.R. Boss^e, D.D. Stadler^f, W.E. Lambert^g, M.R. Lasarev^{g,h}, L.A. Okitunduⁱ, D. Mumba Ngoyi^{j,k}, J.P. Banea^{c,l}, and D.D. Tshala-Katumbayi^{i,k,m,*}

^aDepartment of Biomedical Sciences, University of Kinshasa, Congo-Kinshasa, Democratic Republic of the Congo

^bDepartment of Biology, University of Kinshasa, Congo-Kinshasa, Democratic Republic of the Congo

^cSchool of Public Health, University of Kinshasa, Congo-Kinshasa, Democratic Republic of the Congo

^dDepartment of Psychiatry and Neurology/Ophthalmology, Michigan State University, East Lansing, MI, USA

^eDepartment of Medicine, University of California, San Diego, La Jolla, CA, USA

^fGraduate Programs in Human Nutrition, Oregon Health & Science University, Portland, OR, USA

^gDepartment of Public Health and Preventive Medicine, Oregon Health & Science University, Portland, OR, USA

^hOregon Institute of Occupational Health Sciences, Oregon Health & Science University, Portland, OR, USA

ⁱDepartment of Neurology, University of Kinshasa, Congo-Kinshasa, Democratic Republic of the Congo

^jDepartment of Tropical Medicine, University of Kinshasa, Congo-Kinshasa, Democratic Republic of the Congo

^kInstitut National de Recherches Biomédicales (INRB), Congo-Kinshasa, Democratic Republic of the Congo

^lNational Nutrition Program, Ministry of Health, Congo-Kinshasa, Democratic Republic of the Congo

^mDepartment of Neurology, Oregon Health & Science University, Portland, OR, USA

Abstract

*Corresponding author at: Department of Neurology, Oregon Health and Science University, 3181 Sam Jackson Park Road, Portland, OR 97239, USA. tshalad@ohsu.edu (D.D. Tshala-Katumbayi).

Using a matched case-control design, we sought to determine whether the odds of konzo, a distinct spastic paraparesis associated with food (cassava) cyanogenic exposure in the tropics, were associated with lower cyanide detoxification rates (CDR) and malnutrition. Children with konzo (N = 122, 5–17 years of age) were age- and sex-matched with presumably healthy controls (N = 87) and assessed for motor and cognition performances, cyanogenic exposure, nutritional status, and cyanide detoxification rates (CDR). Cyanogenic exposure was ascertained by thiocyanate (SCN) concentrations in plasma (P-SCN) and urine (U-SCN). Children with a height-for-age z-score (HAZNCHS) < -2 were classified as nutritionally stunted. CDR was measured as time required to convert cyanide to SCN, and expressed as ms/μmol SCN/mg protein or as mmolSCN/ml plasma/min. Mean (SD) U-SCN in children with konzo was 521.9 (353.6) μmol/l and was, significantly higher than 384.6 (223.7) μmol/l in those without konzo. Conditional regression analysis of data for age- and sex- matched case-control pairs showed that konzo was associated with stunting (OR: 5.8; 95% CI: 2.7–12.8; p < 0.01; N = 83 paired groups) and higher U-SCN (OR: 1.1; 95% CI: 1.02–1.20 per 50-μmol increase in U-SCN; p = 0.02; N = 47 paired groups). After adjusting for stunting and U-SCN, 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 CDR. Linear regression analysis indicated a significant association between BOT-2 or KABC-II scores and both the HAZNCHS z-score and the U-SCN concentration, but not the CDR. Our findings provide evidence in support of interventions to remove cyanogenic compounds from cassava prior to human consumption or, perhaps, enhance the detoxification of cyanide in those relying on the cassava as the main source of food.

Keywords

Cassava; Cyanide detoxification; Konzo; Neurotoxicity; Rhodanese; Sulfurtransferases

1. Introduction

Cyanogenic poisoning from consumption of cassava root (tapioca) is associated with neurological deficits, including a disease known as *konzo*, in sub-Saharan Africa (Banea et al., 1992; Tylleskar et al., 1994; Howlett et al., 1990; Cliff et al., 2011; Trolli, 1938; Tshala-Katumbay et al., 2013). Subjects with konzo exhibit a distinct spasticity while walking and running. Fine motor control deficits and exaggerated deep tendon reflexes with or without ankle clonus occur in up to 20% of the general population in konzo-affected areas, suggesting that subclinical forms of cassava neurotoxicity exist (Howlett et al., 1990; Tshala-Katumbay et al., 2002, 2001; Cliff et al., 1999). A recent neuropsychological study revealed cognitive deficits in children with konzo and, to a lesser extent, in those in the same villages but with no paralysis, compared to children from neighboring villages where the disease was not present (Boivin et al., 2013). Whether the motor and cognitive deficits in children living in konzo-affected areas are caused by the same mechanisms has yet to be determined (Boivin et al., 2013; Bumoko et al., 2014; Makila-Mabe et al., 2014). Konzo outbreaks are consistently associated with chronic malnutrition and heavy dietary reliance on insufficiently processed bitter (cyanogenic) cassava as the main food source (Banea et al., 1992; Tylleskar et al., 1994; Cliff et al., 2011; Tshala-Katumbay et al., 2013; Banea et al., 2013, 2014, 2012; Banea-Mayambu et al., 1997; Nzwalo and Cliff, 2011; Mlingi et al.,

1991; Tylleskar et al., 1992, 1991, 1995, 1996, 1993; Ciglenecki et al., 2011; Mozambique, 1984). A substantial body of evidence suggests that neurological insults arise from the ingestion of cassava cyanogens, which then break down in the gut to form neurotoxic compounds such as cyanide and related metabolites (Tshala-Katumbay et al., 2013). While most people from konzo-affected areas rely on the same cyanogenic cassava as the main source of food, only a fraction (i.e., up to 20%) presents with neuropsychological deficits. Of these, varying degrees of severity are observed suggesting that there may be individual factors that dictate the susceptibility to cassava cyanogens and/or their neurotoxic metabolites (Tshala-Katumbay et al., 2013, 2001; Cliff et al., 1999; Cliff and Nicala, 1997). For example, young children and women of child-bearing age are at the highest risk for konzo, but the biologic mechanisms underlying this susceptibility are not understood (Tshala-Katumbay et al., 2013). Poor cyanide detoxification possibly due to poor nutrition, chemical toxicities, or genetic variations notably in sulfurtransferases involved in the detoxification of cyanide has also been hypothesized but not empirically demonstrated (Tshala-Katumbay et al., 2013; Billaut-Laden et al., 2006a, b; Zielinska et al., 2005). In this study, we measured sulfurtransferase detoxification rates of cyanide in human subjects to confirm that the odds of having konzo were associated with poor sulfurtransferase-mediated detoxification of cyanide. These findings underscore the importance of removing cyanogenic compounds from cassava prior to human consumption, and, possibly, implementing strategies to enhance the metabolic detoxification of cyanide in those reliant on cassava as the main source of food.

2. Materials and methods

2.1. Study implementation

We first conducted a survey in August 2012 to assess an ongoing outbreak of konzo in Kahemba, a district with the highest prevalence of the disease in the Democratic Republic of Congo (DRC). Over 3000 children in this district were reported to have konzo in 2011 (Reports DRC Ministry of Health). Interviews with village leaders revealed that the people had endured food shortages on several occasions, notably in times of armed conflict and displacement during the Angola war that forced residents to rely almost exclusively on bitter cassava as their main food source (Okitundu Luwa et al., 2014). During the initial visit to Kahemba, samples of cassava flour from 18 consenting households were collected and found to have cyanide concentrations of 30–200 ppm, well-above the 10 ppm safe limit proposed by the WHO (Joint FAO/OMS report on food contaminants, Rotterdam, 2009). In September 2012, we then designed a case-control study to elucidate the factors associated with the Kahemba konzo outbreak, which appeared to be the largest in the history of the disease (Okitundu Luwa et al., 2014). Neuropsychological findings including demographic, nutritional, and culturally associated risk factors have been previously published (Boivin et al., 2013; Okitundu Luwa et al., 2014).

2.2. Case-control design

Children aged 5–17 years and suspected of having konzo were identified through medical records from the Kahemba primary care clinic, interviews with key informants, and church and radio announcements, as the disease was well known to villagers. To be included in the

study, subjects with konzo 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) onset in less than one week, followed by a non-progressive course in a formerly healthy person; and (3) bilaterally exaggerated knee or ankle reflexes without signs of spinal disease. The severity of the disease was graded as follows: (1) mild—subject able to walk without support; (2) moderate—subject requires support to walk; and (3) severe—subject unable to walk (WHO, 1996). A pool of 122 subjects meeting this case definition was identified for potential participation in a case-control study. Healthy control children as per the interview with the study team neurologist were recruited from the same population; only those children who were residents of Kahemba at the time of the outbreak were eligible to participate as control subjects. Children with a history of an illness that could affect the nervous system, e.g., cerebral malaria, HIV I/II, or HTLV-I/II infections, were excluded. A pool of 87 children suitable as controls was assembled and *m:n* age- and sex-matched to cases. The number of cases and respective matched controls ranged from 1 to 4 in the aforementioned *m:n* matching design.

2.2.1. Ethics statement—Informed consent and child assent were obtained verbally by investigators fluent in Lingala and/or Kikongo, the local spoken languages. Parents who allowed their children to participate in the study were asked to sign a consent form that was kept in the study office. The research protocol, including informed consent and assent procedures, were approved by the Oregon Health & Science University (OHSU) Institutional Review Board and the DRC Ministry of Health.

2.3. Exposure and outcome measurements

2.3.1. Sample collection and storage—A team of laboratory technicians collected samples from people living in the remote and rural district of Kahemba in the DRC. Blood was collected by venipuncture into vacutainer tubes with EDTA and kept at room temperature for ~2 h. The blood was centrifuged at 15,000 rpm for 15 min, and the plasma was transferred to cryotubes and flash-frozen in liquid nitrogen. Urine samples, obtained at the time of blood collection, were also flash frozen in liquid nitrogen. All samples were shipped to Kinshasa, the capital city of the DRC, and stored at – 80 °C until they were shipped to OHSU (Portland, Oregon, USA) on dry ice for biochemical analyses.

2.3.2. Assessment of cyanogenic exposure—Urine samples were analyzed using the urinary SCN picrate kit D1 (Haque and Bradbury, 1999). A color chart was used with 10 shades of color from yellow to brown, corresponding to 0–100 mg thiocyanate/l urine (ppm). The results in ppm were then multiplied by 17.2 to convert to μ mole thiocyanate/l urine. To measure plasma-SCN, 80 μ l of plasma was mixed with 20 μ l of 37% formaldehyde solution (Fisher Scientific). 100 μ l of a ferric nitrate-nitric acid reagent, 1 g Fe(NO₃)₃·9H₂O (Sigma-Aldrich) dissolved in 4 ml HNO₃ (Fisher Scientific) made up to 15 ml with water, was added to the plasma-formaldehyde. Absorbance of the mixture at 414 nm was measured by spectrophotometry and SCN concentration was calculated using a standard curve (Bowler, 1944).

2.3.3. Assessment of stunting—Height-for-age z-score calculated according the US National Center for Health Statistics (HAZNCHS) was used to assess nutritional status (<http://www.cdc.gov/nchs/>). Standing height was measured using a wall-mounted stadiometer; 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://www.cdc.gov/epiinfo/7/index.htm>). Children with a HAZNCHS z-score < - 2 were classified as stunted.

2.3.4. Enzyme assay to determine cyanide detoxification rates—Plasma sulfurtransferase-mediated cyanide detoxification rates were measured according to Sörbo (Sorbo, 1953). Briefly, plasma was incubated for 20 min at 37 °C in 200 mM potassium phosphate buffer, pH 8.6, 25 mM sodium thiosulfate, and 250 mM potassium cyanide. The reaction was stopped by adding 37% formaldehyde, and generated SCN was detected by adding ferric nitrate to form a red product. Absorbance of the final solution was measured at 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 plasma SCN concentration was calculated from standards prepared fresh on the day of assay, and enzyme activity was expressed as $\mu\text{mole SCN}$ formed per minute per mg protein at pH 8.6 and 37 °C. This was later expressed as the number of milliseconds required to detoxify cyanide to yield 1 μmol of SCN per mg of protein [$\text{ms}/(\mu\text{mol}/\text{mg protein})$] in the tested sample to allow cross-species reference to our previous work for future experimental modeling of cyanide (cassava) toxicity or, as the number of millimoles of SCN produced per min in one ml of plasma [$\text{mmolSCN}/(\text{ml plasma}/\text{min})$] after taking into consideration the concentration of protein in each plasma sample. Total plasma protein concentration was quantified using the BCA Protein Assay Kit (Fischer Scientific).

2.3.5. Neurological assessment—Each child's status was recorded as “konzo” or “non-konzo” according to the WHO criteria for the disease, and for those with konzo, disease severity was determined, as defined above. Each child was also assessed using the BOT-2 (Bruininks/Oseretsky Test, 2nd Edition) and KABC-II (Kaufman Assessment Battery for Children, 2nd edition) testing batteries for motor and cognitive performance, respectively. BOT-2 is regarded as one of the most comprehensive and sensitive instruments for motor assessment (Boivin et al., 2010). Testing involves game-like tasks that hold the subject's interest and are not verbally complex. Performance scores are given for fine manual control, manual coordination, body coordination, strength and agility and a final total composite score is calculated. This tool was shown to be adaptable and useful in characterizing specific aspects of motor impairment associated with HIV infection in Ugandan children (Boivin et al., 2010). The KABC battery has been validated in Uganda and used in the DRC prior to this study (Boivin et al., 2010; Boivin and Giordani, 1993; Boivin et al., 1993). The battery measures performances in sequential and simultaneous processing, learning, and planning domains of cognition. Its global mental processing index, the KABC-II score, was used to quantitatively measure cognition.

2.4. Statistical analysis

Disease status (konzo vs. non-konzo) and BOT-2 and KABC-II test scores were the main outcomes. Plasma cyanide detoxification rate (CDR), P-SCN and U-SCN, and nutritional status (HAZNCHS z-scores or stunting status Yes/No) were the main predictors. Initial analyses consisted of Student *t* test, ANOVA, Mann-Whitney, and Kruskal-Wallis tests to compare key characteristics of exposure to cyanogenic compounds, nutritional status, CDR, or neurocognitive performance. Thereafter, a multivariable analysis used a conditional logistic regression to determine the odds of having konzo in the *m:n* age- and sex-matched case-control groups in relation to the aforementioned predictors. Linear regression determined whether the quantitative measures of motor proficiency and neurocognitive performance, i.e. BOT-2 and KABC-II scores, were associated with the same predictors. 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. STATA software (version 11.2) was used for all analyses, with significance set at $p = 0.05$.

3. Results

3.1. Age and sex distribution

We enrolled 122 subjects affected by konzo [aged 4.3–17.6 years; mean (SD): 8.7 (2.6) years] and 87 control subjects [aged 4.7–15.4 years; mean (SD): 9.1 (2.6) years]. Of those affected by konzo, 65 (53.3%) were boys and 57 (46.7%) were girls, compared to the control group comprised of 52 (59.8%) boys and 35 (40.2%) girls.

3.2. Motor and cognition deficits

Of the 122 children with konzo, 91 (74.6%) had a mild form of the disease while 18 (14.7%) and 13 (10.7%) had a severe or moderate form of konzo, respectively. Disease duration from 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 score for cognition was 58 (52–65) in children with konzo compared to 60 (54–68) in those not affected by the disease ($p < 0.03$, Mann-Whitney test). Severely affected subjects performed worse on both the BOT-2 and KABC-II testing batteries. Median scores changed in accordance with disease severity ($p < 0.01$, Kruskal-Wallis test) (Table 1).

3.3. Nutritional status in relation to konzo

The HAZNCHS z-score was significantly lower in children with konzo [mean (SD): –3.4 (1.4)] compared to those without konzo [mean (SD): –2.3 (1.8)] ($p < 0.05$, Student *t* test). No effect of sex was seen on the z-score irrespective of konzo status. 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 children (17/18) with severe konzo were stunted.

3.4. Cyanogenic exposure and cyanide detoxification rates

The U-SCN and P-SCN concentrations ranged from 17.2 to 1720 and 64–426 $\mu\text{mol/l}$, respectively. The mean (SD) and median (IQR) P-SCN were 228.1 (69.8) and 224.5 (179.0–279.4) $\mu\text{mol/l}$, respectively. For U-SCN, the overall mean (SD) and median (IQR) were 474.2 (320.6) and 344 (144–688) $\mu\text{mol/l}$, respectively. Overall, U-SCN positively correlated with P-SCN (Spearman $r = 0.27$, $p < 0.01$). With respect to the disease status, children with konzo had U-SCN and P-SCN ranging from 17.2 to 1720 and 64–426 $\mu\text{mol SCN/l}$, respectively, compared to controls whose U-SCN and P-SCN ranged from 34.4 to 1032 and 69–410 $\mu\text{mol SCN/l}$, respectively. U-SCN positively correlated with P-SCN (Spearman $r = 0.28$, $p < 0.01$) in the konzo groups but not in the non-konzo group (Spearman $r = 0.25$, $p = 0.11$). With respect to nutritional status, U-SCN concentrations ranged from 17.2 to 1720 $\mu\text{mol SCN/l}$ in the stunted group compared to 172–1032 $\mu\text{mol SCN/l}$ in the non-stunted group, and P-SCN ranged from 64 to 426 $\mu\text{mol SCN/l}$ in the stunted group compared to 117–425 $\mu\text{mol SCN/l}$ in the non-stunted group. U-SCN was positively correlated with P-SCN (Spearman $r = 0.26$, $p = 0.01$) in the stunted group but not in the non-stunted group (Spearman $r = 0.30$, $p = 0.11$).

Overall, the CDR ranged from 78.9 to 1670 $\text{ms}/(\mu\text{molSCN}/\text{mg protein})$ or, equivalently, from 2.5 to 30.1 $\text{mmolSCN}/(\text{ml plasma}/\text{min})$. The mean (SD) CDR was 420.1 (201) $\text{ms}/(\mu\text{molSCN}/\text{mg protein})$ or, equivalently, 12.0 (4.9) $\text{mmolSCN}/(\text{ml plasma}/\text{min})$. The median (IQR) was 383.6 (278.1–519.9) $\text{ms}/(\mu\text{molSCN}/\text{mg protein})$ or, equivalently, 11.1 (8.3–14.5) $\text{mmolSCN}/(\text{ml plasma}/\text{min})$. Stunted children had CDRs that ranged from 78.9 to 1670 $\text{ms}/(\mu\text{molSCN}/\text{mg protein})$ or 2.5–30.1 $\text{mmolSCN}/(\text{ml plasma}/\text{min})$ compared to 134.1–1053 $\text{ms}/(\mu\text{molSCN}/\text{mg protein})$ or 4.7–26.3 $\text{mmolSCN}/(\text{ml plasma}/\text{min})$ in those who were apparently well nourished (Table 2).

Children with konzo had CDRs ranging from 78.9 to 1670 $\text{ms}/(\mu\text{mol SCN}/\text{mg protein})$ or 2.5–26.3 $\text{mmol SCN}/(\text{ml plasma}/\text{min})$ compared to 104.8–1053 $\text{ms}/(\mu\text{mol SCN}/\text{mg protein})$ or 4.3–30.1 $\text{mmol SCN}/(\text{ml plasma}/\text{min})$ in those without konzo. No significant correlation was found between CDR and U-SCN or P-SCN irrespective of stunting or disease status. The mean (SD) and median (IQR) CDR, U-SCN and P-SCN in relation to disease status are presented in Table 3a and b.

3.5. Neurological deficits in relation to exposure, nutritional status, and cyanide detoxification rates

Conditional logistic regression analyses showed that having konzo was associated with stunting, the U-SCN concentration, and CDR as measured by the amount of SCN produced per min per ml of plasma after adjusting for U-SCN (Table 4). Analysis of matched case-control pairs with a complete set of data showed 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- $\text{mmol SCN}/(\text{ml plasma}/\text{min})$ increase in the cyanide detoxification rate. 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 in the U-SCN concentration in the same association model (Table 5).

Linear regression analysis indicated a significant association between BOT-2 or KABC-II scores and both the HAZNCHS z-score and the U-SCN concentration, but not the CDR. In non-parametric analyses, BOT-2 scores correlated positively with the HAZNCHS z-score (Spearman $r = 0.44$, $p < 0.01$) in the konzo group but not in the non-konzo group (Spearman $r = 0.25$, $p > 0.05$). A similar trend was seen for the KABC-II score, which correlated positively with the HAZNCHS z-score (Spearman $r = 0.32$, $p = 0.00$) in the konzo group but not in the non-konzo group (Spearman $r = 0.22$, $p > 0.05$). Both the BOT-2 and KABC-II scores, however, failed to correlate with CDR.

4. Discussion

We report for the first time plasma cyanide detoxification rates in children reliant on cyanogenic cassava as the main source of food. Children with konzo required up to 1670 ms to produce one μmol of SCN/mg protein in their plasma, which is about one-third the rate of that observed in non-human primates *macaca fascicularis* or two-thirds the rate of that observed in rodents. Detoxification rates in children without konzo were up to 1050 ms/ μmol SCN/mg protein, which are equivalent to the rates reported for rodents (Kimani et al., 2014). Despite the lack of statistical significance, stunted children may detoxify cyanide faster than those who are not stunted. In addition, their plasma thiocyanate concentration positively correlates, as in children with konzo, with that of urinary thiocyanate. This observation appears to be consistent with previous studies that suggested that protein catabolism is enhanced in subjects on the cassava-based cyanogenic diet to provide sulfur for the detoxification of cyanide, which in turn possibly lead to stunting (Tshala-Katumbay and Spencer, 2007; Banea-Mayambu et al., 2000). A consequence of this hypothesis is that subjects on a cyanogenic diet notably those stunted or affected by konzo 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 (Makila-Mabe et al., 2014; Tshala-Katumbay and Spencer, 2007).

After adjusting for stunting and urinary concentrations of SCN, we found that the odds of having konzo was reduced by 63% for each five-unit increase in cyanide detoxification rate. This finding appears to be consistent with changes in CDR by disease status and severity, which indicate that severely affected children tend to have lower detoxification rates. Reverse causation, however, remains possible since children with severe konzo may disproportionately suffer from malnutrition and therefore, present with poor detoxification capabilities (Tshala-Katumbay et al., 2013). The association between konzo and CDR in $\text{mmol SCN}/(\text{ml plasma}/\text{min})$, which was calculated taking into account the total protein concentrations in plasma, but not with CDR expressed in $\text{ms}/(\mu\text{mol SCN}/\text{mg protein})$ underscores the importance of plasma total protein concentration in assessing cyanide detoxification capabilities. However, further insights into this proposal may only be gained from in-depth proteomic analyses. This would allow characterization of the exact identity and differential expression of proteins of interest.

We failed to demonstrate a linear correlation between CDR and neurological deficits assessed by BOT-2 and KABC-II testing. Although a non-linear relationship may exist, our findings illustrate 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.

Several limitations should be considered in the interpretation of our findings. First, the non-random selection of subjects may limit generalizability. Additionally, the small sample size would not allow in-depth analyses of the data with respect to the severity of konzo, though children moderately affected by konzo were observed to detoxify cyanide better than their counterparts (perhaps due to unknown mechanisms of adaptation). Other study limitations include non-concomitant measures of exposure, detoxification rates, and neurological deficits including a persistent disease (konzo) state that occurred several months prior to the study. In addition, we have not explored non-enzymatic processes of cyanide detoxification, and a case-control design does not allow ruling out reverse causation. Nevertheless, we have shown that children with konzo remain at risk for toxic injury due to poor cyanide detoxification capabilities. This is mostly worrisome since the combination of poor cyanide detoxification and higher U-SCN in children with konzo does suggest that they may experience higher exposure to cassava cyanogens compared to presumably healthy controls. Thus, prevention of the effects of cassava neurotoxicity, including konzo, may require both a reduction in cyanide exposure through safer processing of cassava and strategies to enhance the detoxification of cyanide in those relying on cassava as the main source of food.

Our study findings support a theoretical framework that highlights further risks for cassava neurotoxicity in subjects affected by konzo as they appear less competent in cyanide detoxification mechanisms. Longitudinal studies may allow measuring risks for recurrent toxic injury in relation to cyanide detoxification capabilities. In this respect, field-relevant (point-of-care) diagnostic tools to monitor risks in relation to cyanide exposure and metabolism are needed. Whether lower detoxification rates are mediated through genetics, deficiencies in select nutrients, or metabolic changes induced by cyanide or related metabolites such as the cyanide metabolite and carbamoylating agent cyanate has yet to be determined (Tshala-Katumbay et al., 2013; Makila-Mabe et al., 2014; Billaut-Laden et al., 2006a, b; Kimani et al., 2014; Banea-Mayambu et al., 2000; Kraus and Kraus, 2001). The potential for sustained and repeated neurotoxic injuries in subjects living in konzo areas warrants studies to assess the global burden of disease associated with chronic dietary reliance on cyanogenic cassava as the main source of food. Experimental modeling to determine strategies to enhance the detoxification of cyanide may offer new lines of public health interventions to prevent the neurotoxic effects of cassava.

Acknowledgements

We would like to express our gratitude to community of Kahemba and the USA-DRC konzo research team for their participation in the study. This research was funded by the National Institutes of Health through the NIEHS/FIC grant R01ES019841.

References

- Banea M, Bikangi N, Nahimana G, Nunga M, Tylleskar T, Rosling H. [High prevalence of konzo associated with a food shortage crisis in the Bandundu region of zaire]. *Ann. Soc. Belg. Med. Trop.* 1992; 72(4):295–309. [PubMed: 1292426]

- Banea JP, Nahimana G, Mandombi C, Bradbury JH, Denton IC, Kuwa N. Control of konzo in DRC using the wetting method on cassava flour. *Food Chem. Toxicol.* 2012; 50(5):1517–1523. [PubMed: 22342647]
- Banea JP, Bradbury JH, Mandombi C, Nahimana D, Denton IC, Kuwa N, et al. Control of konzo by detoxification of cassava flour in three villages in the Democratic Republic of Congo. *Food Chem. Toxicol.* 2013; 60:506–513. [PubMed: 23941775]
- Banea JP, Bradbury JH, Mandombi C, Nahimana D, Denton IC, Kuwa N, et al. Effectiveness of wetting method for control of konzo and reduction of cyanide poisoning by removal of cyanogens from cassava flour. *Food Nutr. Bull.* 2014; 35(1):28–32. [PubMed: 24791576]
- Banea-Mayambu JP, Tylleskar T, Gitebo N, Matadi N, Gebre-Medhin M, Rosling H. Geographical and seasonal association between linamarin and cyanide exposure from cassava and the upper motor neurone disease konzo in former Zaire. *Trop. Med. Int. Health.* 1997; 2(12):1143–1151. [PubMed: 9438470]
- Banea-Mayambu JP, Tylleskar T, Tylleskar K, Gebre-Medhin M, Rosling H. Dietary cyanide from insufficiently processed cassava and growth retardation in children in the Democratic Republic of Congo (formerly Zaire). *Ann. Trop. Paediatr.* 2000; 20(1):34–40. [PubMed: 10824211]
- Billaut-Laden I, Allorge D, Crunelle-Thibaut A, Rat E, Cauffiez C, Chevalier D, et al. Evidence for a functional genetic polymorphism of the human thiosulfate sulfurtransferase (Rhodanese), a cyanide and H₂S detoxification enzyme. *Toxicology.* 2006a; 225(1):1–11. [PubMed: 16790311]
- Billaut-Laden I, Rat E, Allorge D, Crunelle-Thibaut A, Cauffiez C, Chevalier D, et al. Evidence for a functional genetic polymorphism of the human mercaptopyruvate sulfurtransferase (MPST), a cyanide detoxification enzyme. *Toxicol. Lett.* 2006b; 165(2):101–111. [PubMed: 16545926]
- Boivin MJ, Giordani B. Improvements in cognitive performance for schoolchildren in Zaire, Africa, following an iron supplement and treatment for intestinal parasites. *J. Pediatr. Psychol.* 1993; 18(2): 249–264. [PubMed: 8492277]
- Boivin MJ, Giordani B, Ndanga K, Maky MM, Manzeki KM, Ngunu N, et al. Effects of treatment for intestinal parasites and malaria on the cognitive abilities of schoolchildren in Zaire, Africa. *Health Psychol.* 1993; 12(3):220–226. [PubMed: 8500452]
- Boivin MJ, Ruel TD, Boal HE, Bangirana P, Cao H, Eller LA, et al. HIV-subtype A is associated with poorer neuropsychological performance compared with subtype D in antiretroviral therapy-naïve Ugandan children. *AIDS.* 2010; 24(8):1163–1170. [PubMed: 20425886]
- Boivin MJ, Okitundu D, Makila-Mabe Bumoko G, Sombo MT, Mumba D, Tylleskar T, et al. Neuropsychological effects of konzo: a neuromotor disease associated with poorly processed cassava. *Pediatrics.* 2013; 131(4):e1231–9. [PubMed: 23530166]
- Bowler RG. The determination of thiocyanate in blood serum. *Biochem. J.* 1944; 38(5):385–388. [PubMed: 16747819]
- Bumoko GM, Sombo MT, Okitundu LD, Mumba DN, Kazadi KT, Tamfum-Muyembe JJ, et al. Determinants of cognitive performance in children relying on cyanogenic cassava as staple food. *Metab. Brain Dis.* 2014; 29(2):359–366. [PubMed: 24481810]
- Ciglencecki I, Eyema R, Kabanda C, Taafo F, Mekaoui H, Urbaniak V. Konzo outbreak among refugees from Central African Republic in eastern region, Cameroon. *Food Chem. Toxicol.* 2011; 49(3): 579–582. [PubMed: 20538034]
- Cliff J, Nicala D. Long-term follow-up of konzo patients. *Trans. R. Soc. Trop. Med. Hyg.* 1997; 91(4): 447–449. [PubMed: 9373651]
- Cliff J, Nicala D, Saute F, Givragy R, Azambuja G, Taela A, et al. Ankle clonus and thiocyanate, linamarin, and inorganic sulphate excretion in school children in communities with Konzo, Mozambique. *J. Trop. Pediatr.* 1999; 45(3):139–142. [PubMed: 10401190]
- Cliff J, Muquingue H, Nhassico D, Nzwalo H, Bradbury JH. Konzo and continuing cyanide intoxication from cassava in Mozambique. *Food Chem. Toxicol.* 2011; 49(3):631–635. [PubMed: 20654676]
- Haque MR, Bradbury JH. Simple method for determination of thiocyanate in urine. *Clin. Chem.* 1999; 45(9):1459–1464. [PubMed: 10471648]
- Howlett WP, Brubaker GR, Mlingi N, Rosling H. Konzo, an epidemic upper motor neuron disease studied in Tanzania. *Brain.* 1990; 113(Pt 1):223–235. [PubMed: 2302534]

- Kimani S, Moterroso V, Morales P, Wagner J, Kipruto S, Bukachi F, et al. Cross-species and tissue variations in cyanide detoxification rates in rodents and non-human primates on protein-restricted diet. *Food Chem. Toxicol.* 2014; 66:203–209. [PubMed: 24500607]
- Makila-Mabe BG, Kikandau KJ, Sombo TM, Okitundu DL, Mwanza JC, Boivin MJ, et al. Serum 8,12-iso-iPF₂alpha-VI isoprostane marker of oxidative damage and cognition deficits in children with konzo. *PLoS One.* 2014; 9(9):e107191. [PubMed: 25222616]
- Mlingi N, Kimatta S, Rosling H. Konzo a paralytic disease observed in southern Tanzania. *Trop. Doct.* 1991; 21(1):24–25.
- Mozambique, MoH. Mantakassa: an epidemic of spastic paraparesis associated with chronic cyanide intoxication in a cassava staple area in Mozambique. II. Nutritional factors and hydrocyanic content of cassava products. *Bull. WHO.* 1984; 62:485–492. [PubMed: 6088100]
- Nzwalo H, Cliff J. Konzo: from poverty, cassava, and cyanogen intake to toxico-nutritional neurological disease. *PLoS Negl. Trop. Dis.* 2011; 5(6):e1051. [PubMed: 21738800]
- Okitundu Luwa EAD, Bumoko Makila-Mabe G, Ayanne MT, Kikandau JK, Mashukano N, Kazadi Kayembe T, et al. [Persistence of konzo epidemics in Kahemba, Democratic Republic of Congo: phenomenological and socioeconomic aspects]. *Pan Afr. Med. J.* 2014; 18:213. [PubMed: 25419339]
- Sorbo B. Crystalline rhodanese. *Acta Chem. Scand.* 1953; 7:1137–1145.
- Trolli, G. Paraplégie spastique épidémique, Konzo des indigènes du Kwango. In: Trolli, G., editor. Résumé des observations réunies, au Kwango, au sujet de deux affections d'origine indéterminée. Fonds Reine Elisabeth; Brussels: 1938. p. 1-36.
- Tshala-Katumbay DD, Spencer PS. Chapter 18 toxic disorders of the upper motor neuron system. *Handb. Clin. Neurol.* 2007; 82:353–372. [PubMed: 18808903]
- Tshala-Katumbay D, Eeg-Olofsson KE, Tylleskar T, Kazadi-Kayembe T. Impairments, disabilities and handicap pattern in konzo—a non-progressive spastic para/tetraparesis of acute onset. *Disabil. Rehabil.* 2001; 23(16):731–736. [PubMed: 11732562]
- Tshala-Katumbay D, Eeg-Olofsson KE, Kazadi-Kayembe T, Tylleskar T, Fallmar P. Analysis of motor pathway involvement in konzo using transcranial electrical and magnetic stimulation. *Muscle Nerve.* 2002; 25(2):230–235. [PubMed: 11870691]
- Tshala-Katumbay D, Mumba N, Okitundu L, Kazadi K, Banea M, Tylleskar T, et al. Cassava food toxins, konzo disease, and neurodegeneration in sub-Saharan Africans. *Neurology.* 2013; 80(10):949–951. [PubMed: 23460617]
- Tylleskar T, Banea M, Bikangi N, Fresco L, Persson LA, Rosling H. Epidemiological evidence from Zaire for a dietary etiology of konzo, an upper motor neuron disease. *Bull. World Health Organ.* 1991; 69(5):581–589. [PubMed: 1959159]
- Tylleskar T, Banea M, Bikangi N, Cooke RD, Poulter NH, Rosling H. Cassava cyanogens and konzo, an upper motoneuron disease found in Africa. *Lancet.* 1992; 339(8787):208–211. [PubMed: 1346173]
- Tylleskar T, Howlett WP, Rwiza HT, Aquilonius SM, Stalberg E, Linden B, et al. Konzo: a distinct disease entity with selective upper motor neuron damage. *J. Neurol. Neurosurg. Psychiatry.* 1993; 56(6):638–643. [PubMed: 8509777]
- Tylleskar T, Legue FD, Peterson S, Kpizingui E, Stecker P. Konzo in the Central African Republic. *Neurology.* 1994; 44(5):959–961. [PubMed: 8190305]
- Tylleskar T, Banea M, Bikangi N, Nahimana G, Persson LA, Rosling H. Dietary determinants of a non-progressive spastic paraparesis (Konzo): a case-referent study in a high incidence area of Zaire. *Int. J. Epidemiol.* 1995; 24(5):949–956. [PubMed: 8557452]
- Tylleskar T, Banea M, Bottiger B, Thorstensson R, Biberfeld G, Rosling H. Konzo an epidemic spastic paraparesis in Africa, is not associated with antibodies to HTLV-I, HIV, or HIV gag-encoded proteins. *J. Acquir. Immune Defic. Syndr. Hum. Retrovirol.* 1996; 12(3):317–318. [PubMed: 8673538]
- WHO. Konzo: a distinct type of upper motor neuron disease. *Wkly. Epidemiol. Rec.* 1996; 71:225–232. [PubMed: 8718826]

- Zielinska E, Zubowska M, Misiura K. Role of GSTM1, GSTP1, and GSTT1 gene polymorphism in ifosfamide metabolism affecting neurotoxicity and nephrotoxicity in children. *J. Pediatr. Hematol. Oncol.* 2005; 27(11):582–589. [PubMed: 16282887]
- Kraus LM, Kraus AP Jr. Carbamoylation of amino acids and proteins in uremia. *Kidney Int. Suppl.* 2001; 78:S102–S107. [PubMed: 11168993]

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 disease status and severity. However, significant differences were mostly noted between severely affected children and those with no or mild konzo (BOT-2 testing) and between severely affected children and the remaining groups (KABC-II testing) ($p < 0.01$, Kruskal-Wallis test).

Table 2

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

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 [ms/(μ molSCN/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 (μ mol/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 (μ mol/l)	N = 108	N = 34
Mean (SD)	474.6 (333.4)	470.5 (288.1)
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 [ms/(μ molSCN/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 (μ mol/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 (μ mol/l)	N = 94	N = 50
Mean (SD)	521.9 (353.6)	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 [ms/(μ molSCN/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 (μ mol/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 (μ mol/l)	N = 68	N = 9	N = 17
Mean (SD)	501.1 (340.3)	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).

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 [ms/(μ mol 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 (μ mol/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 (μ mol 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

The disease status konzo was associated with stunting, U-SCN concentration, and CDR as measured by the amount of SCN produced per min per ml of plasma after adjusting for U-SCN.

Table 5

Odds of konzo in relation to cyanide detoxification rates (CDR) and plasma and urinary thiocyanate (SCN) concentrations in *m:n* matched case-controls 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

The odds of konzo were reduced by 63% for each 5-mmol SCN/(ml plasma/min) increase in the cyanide detoxification rate (OR: 0.37; 95%CI: 0.15–0.89) but increased by 185% with stunting (OR: 2.85; 95%CI: 1.0–8.3) or 20% for each 50-μmol increase in the U-SCN concentration (OR: 1.2; 95%CI: 1.05–1.37).