Research Article

Fumonisin B_1 as a Urinary Biomarker of Exposure in a Maize Intervention Study Among South African Subsistence Farmers

Liana van der Westhuizen¹, Gordon S. Shephard¹, Hester M. Burger¹,², John P. Rheeder¹, Wentzel C.A. Gelderblom¹,², Christopher P. Wild³, and Yun Yun Gong⁴*

Abstract

Background: The consumption of maize highly contaminated with carcinogenic fumonisins has been linked to high oesophageal cancer rates. The aim of this study was to validate a urinary fumonisin B_1 (UFB_1) biomarker as a measure of fumonisin exposure and to investigate the reduction in exposure following a simple and culturally acceptable intervention.

Methods: At baseline home-grown maize, maize-based porridge, and first-void urine samples were collected from female participants (n = 22), following their traditional food practices in Centane, South Africa. During intervention the participants were trained to recognize and remove visibly infected kernels, and to wash the remaining kernels. Participants consumed the porridge prepared from the sorted and washed maize on each day of the two-day intervention. Porridge, maize, and urine samples were collected for FB_1 analyses.

Results: The geometric mean (95% confidence interval) for FB_1 exposure based on porridge (dry weight) consumption at baseline and following intervention was 4.84 (2.87–8.14) and 1.87 (1.40–2.51) mg FB_1/kg body weight/day, respectively, (62% reduction, P < 0.05). UFB_1C, UFB_1 normalized for creatinine, was reduced from 470 (295–750) at baseline to 279 (202–386) pg/mg creatinine following intervention (41% reduction, P = 0.06). The UFB_1C biomarker was positively correlated with FB_1 intake at the individual level (r = 0.4972, P < 0.01). Urinary excretion of FB_1 was estimated to be 0.075% (0.054%–0.104%) of the FB_1 intake.

Conclusion: UFB_1 reflects individual FB_1 exposure and thus represents a valuable biomarker for future fumonisin risk assessment.

Impact: The simple intervention method, hand sorting and washing, could positively impact on food safety and health in communities exposed to fumonisins. Cancer Epidemiol Biomarkers Prev; 20(3); 483–9. ©2011 AACR.

Introduction

Fumonisins are a group of mycotoxins; mainly produced by Fusarium spp. as secondary metabolites, of which fumonisin B_1, B_2, and B_3 (FB_1, FB_2, and FB_3) are the most prevalent (1). Fumonisins are carcinogenic to rodents, and have been associated with oesophageal and liver cancer in subsistence maize farming communities around the world, as well as occurrence of neural tube defects (2, 3, 4, 5). There are numerous fumonisin analogues of which FB_1 is the most abundant naturally occurring fumonisin on maize and maize-based food worldwide and was classified as "possibly carcinogenic to humans" by the International Agency for Research on Cancer (6). The Joint FAO/WHO Expert Committee on Food Additives (JECFA) has determined a group provisional maximum tolerable daily intake (PMTDI) for FB_1, FB_2, and FB_3 alone or in combination, of 2 µg/kg body weight/day (7).

In many Sub-Saharan countries, where both maize contamination and maize consumption are high, regulatory mechanisms to control mycotoxin levels, including fumonisin, are either lacking or are not enforced (8). Therefore, reducing exposure levels by intervention, specifically those based on simple low-cost measures acceptable to these communities, becomes critical to protect the population at greatest risk (9). Such an approach gave promising results with respect to aflatoxins in groundnuts (10).

Conventional assessment of mycotoxin exposure, probable daily intake (PDI), is based on the amount of...
food consumed and the contamination level of the food (11). Accuracy of the PDI can be improved by determining food consumption and the level of the mycotoxin in the food on an individual basis (12). The quality of food consumption information in the subsistence community setting depends on the validity of the assessment methods and measuring of food on the plate prior to and after food consumption, a labour-intensive activity (11). Utilization of validated biomarkers of exposure, taking advantage of measuring individual internal dose, would not require measurement of consumption or contamination level of the food and thus promises to be more accurate than estimations by other assessment methods (13). Aflatoxin, deoxynivalenol, and ochratoxin A, urinary biomarkers, as well as the aflatoxin-albumin adduct in blood, have been implemented in human studies (14–16). The aflatoxin biomarker applications in human health and intervention studies have demonstrated the value of mycotoxin-related biomarkers (13).

Various animal studies have successfully investigated the sphinganine/sphingosine ratio as a biomarker of fumonisin exposure (11, 17). The sphingolipid bases, sphinganine and sphingosine, as well as their ratio, have also been investigated in several human studies in blood and urine, but could not be correlated with fumonisin exposure (11, 12, 18). A recent study has reported a high-performance liquid chromatography–mass spectrometry (HPLC-MS) method for urinary fumonisin B₁ (UFB₁), which was sufficiently sensitive to be positively correlated with fumonisin exposure in a Mexican population consuming different amounts of maize-based tortillas (19).

In a previous study, customary sorting and washing of maize kernels as food preparation procedures were optimized under laboratory-controlled conditions to achieve optimal fumonisin reduction in contaminated home-grown maize (20). These simple and culturally acceptable food preparation practices reduced fumonisin exposure as assessed by food intake profiles and fumonisin food analysis in a subsistence farming community residing in a high oesophageal cancer incidence area (21). The aim of this study was to validate the UFB₁ biomarker and confirm the reduction in fumonisin exposure at an individual level.

Material and Methods

Participants

The study was approved by the Ethics Committee of the Medical Research Council of South Africa. Following informed consent, apparently healthy females (n = 22) aged between 20 and 70 who prepared maize-based meals from home-grown maize, were recruited from the Centane magisterial district, Eastern Cape Province, South Africa.

Baseline phase of the field study

At baseline, participants consumed their customarily prepared maize-based food (~0.5 kg porridge) twice daily for 2 consecutive days (Table 1). Porridge samples (~0.5 kg) were collected on both days for FB₁ analyses. Morning first-void urine samples were collected from each participant on the subsequent days for FB₁ biomarker analysis. The first-void urine collections were done approximately 12 hours after the participants consumed the last porridge meal. Dietary recall questionnaires were recorded to determine maize intake (dry weight) in the 24 hours prior to urine collection (21). Table 1. Both the baseline and intervention phases of the study were conducted over 3 consecutive days for each participant. Morning first-void urine samples were collected from the participant individually on each day following the twice daily consumption of the porridge. The training was conducted following the completion of the baseline phase preceding the intervention phase of the study.

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>Training</th>
<th>Intervention</th>
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<tbody>
<tr>
<td>Day 1</td>
<td>Consume porridge</td>
<td>Collect first-void urine</td>
<td>Collect first-void urine</td>
</tr>
<tr>
<td>Day 2</td>
<td>Consume porridge</td>
<td>Collect first-void urine</td>
<td>Collect first-void urine</td>
</tr>
<tr>
<td>Day 3</td>
<td>Sorting/washing of maize</td>
<td>Collect sorted/washed maize</td>
<td>Collect first-void urine</td>
</tr>
<tr>
<td>Day 4</td>
<td>Sorting/washing of maize</td>
<td>Collect sorted/washed maize</td>
<td>Collect first-void urine</td>
</tr>
<tr>
<td>Day 5</td>
<td>Consume porridge</td>
<td>Collect first-void urine</td>
<td>Collect first-void urine</td>
</tr>
<tr>
<td>Day 6</td>
<td>Consume porridge</td>
<td>Collect first-void urine</td>
<td>Collect first-void urine</td>
</tr>
<tr>
<td>Day 7</td>
<td>Consume porridge</td>
<td>Collect first-void urine</td>
<td>Collect first-void urine</td>
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</tbody>
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samples (~5 kg) were collected from each participant of which subsamples (~0.5 kg) were retained for FB1 analyses. The remaining kernels were pooled, thoroughly mixed, divided into 4-kg batches (n = 22), and set aside for the intervention study.

**Intervention phase of the field study**

Participants were trained to recognize infected kernels (most likely to be contaminated) and on the washing procedure (10 minutes) for the remaining maize kernels. Following training, each participant first removed visibly infected kernels and subjected the remaining maize kernels to a washing step. Subsamples of the sorted and washed maize of each participant were collected for FB1 analysis and the remainder of the maize kernels pooled for porridge preparation. On the first day of the intervention phase porridge was prepared and weighed as 0.5 kg portions, which were consumed as 2 separate meals (midday and evening) by the participants. First-void urine was collected on the following morning and 24-hour dietary recall questionnaires completed as described previously. The procedure was repeated on the second day of the intervention.

All the maize and porridge samples were stored at 4°C and −20°C, respectively prior to FB1 analysis at the PROMEC Unit. Urine samples were stored at −20°C and sent on dry ice for FB1 analysis at the University of Leeds.

**Analyses**

The maize and porridge samples were analyzed for FB1 following solid phase extraction clean-up, derivatization with o-phthalaldehyde, and detection by fluorescence HPLC (21, 22). The urine samples were cleaned-up by solid-phase extraction and analyzed for FB1 by HPLC-MS with a limit of detection (LOD) of 20 pg/mL (19). Urinary creatinine was determined according to the alkaline-picrate method (23) with minor modifications to adapt to a 96-well plate format. Inter-individual variation in urine concentration was normalized by utilization of urinary creatinine. The biomarker data are presented as both pg FB1/mL urine (UFB1) and pg FB1/mg creatinine (UFB1C).

**Probable daily intake assessment**

Porridge (dry weight) consumption was assessed by 24-hour dietary recall questionnaires utilizing full-scale photographs of small, medium, and large porridge portions. Individual maize intakes (dry weight, g/day) were estimated using the local maize porridge recipes and by assigning specific weights to the portions sizes (21). Individual fumonisin PDI was assessed as FB1 level in the porridge (dry weight) consumed by each participant during the baseline and intervention phases of the study.

**FB1 excretion in urine**

The 24-hour urine output was estimated by assuming that the first-void urine volume represented an 8-hour collection. The urinary FB1 excretion was calculated as follows:

\[
\text{Excretion (\%)} = \frac{\text{UFB1 (pg/mL) \times [8-hour urine output \times 3] (mL/day) \times 100}}{\text{PDI (pg/8 hour body weight/day) \times body weight (kg) \times 10^6}}
\]

**Statistical analysis**

Individual FB1 PDI and UFB1 were assessed as the mean of 2 days. UFB1C data was not normally distributed and was natural log-transformed to facilitate data analysis. Mean FB1 levels in urine and maize were expressed geometrically (95% confidence interval) unless otherwise stated. Student’s t test was used to compare the FB1 exposure at baseline and following intervention. Correlation and regression analyses were performed to examine the association between the PDI and UFB1 or UFB1C levels. Urine samples below the LOD were assigned a value of half the LOD. Multiple linear regression modeling was used to investigate the independent contribution of FB1 PDI and age to the biomarker levels. STATA9.0 (STATACorp) and Number Cruncher Statistical Systems (NCSS) 2004 Dawson Edition (NCSS) were used for the analysis.

**Results**

**Participants**

The mean age of the participants was 42 years (range 20–70), their mean body weight was 65 (47–127) kg and their mean maize consumption (dry weight) at baseline was 0.34 (0.28–0.40) kg/day and following intervention it was 0.38 (0.31–0.47) kg/day as reported in the previous study (20). The mean FB1 level in home-grown maize at baseline [1.16 (0.84–1.56) mg/kg] was significantly reduced (84%, P < 0.05) following the sorting and washing of the kernels during intervention [0.19 (0.14–0.25) mg/kg]. The FB1 represented 72% of the total fumonisins (FB1 + FB2 + FB3) in the maize (FB2 and FB3 levels were reported in a previous study; ref. 20). The porridge at baseline had a mean FB1 of 0.89 (0.55–1.44) mg/kg compared with 0.32 (0.27–0.37) mg/kg following intervention (64% reduction, P < 0.05; Table 2).

**PDI assessment**

The mean PDI of FB1 at baseline was 4.84 (2.87–8.14) μg/kg body weight/day and following intervention the PDI was significantly reduced (62%, P < 0.05) to 1.87 (1.40–2.51) μg/kg body weight/day (Table 2). The mean FB1 exposure at baseline exceeded the JECFA recommended PMTDI (2 μg/kg body weight/day), and was below this PMTDI following intervention. Before intervention 15 of 21 (71%) participants had PDIs exceeding the recommended PMTDI, whereas this frequency was reduced to 10 of 19 (53%) following intervention (p > 0.05). However, FB1 represented only 72% of the total fumonisins in the maize, and the PMTDI determined...
by JECFA was based on FB$_1$, FB$_2$, and FB$_3$, alone or in combination.

**Urine**

At baseline 43 of 44 (98%) and following intervention 40 of 42 (96%) of the urine samples had FB$_1$ above the LOD. Following intervention, the mean UFB$_1$ was reduced from 225 (144–350) to 109 (85–138) pg/mL, a 52% reduction ($P = 0.02$; Table 2). Following normalization with urinary creatinine, UFB$_1$C at baseline and following intervention was 470 (295–750) and 279 (202–386) pg/mg creatinine, respectively, (41% reduction, $P = 0.06$). Individual FB$_1$ intake to UFB$_1$C at baseline and intervention combined is significantly correlated ($r = 0.4972$, $P < 0.01$). In addition, the linear regression model is better fitted when age, a positive contributor (regression coefficient 0.0227, $P < 0.01$), is introduced as the older women were apparently exposed to higher FB$_1$ levels.

**FB$_1$ excretion in urine**

The urine output as well as the urinary excretion was similar at baseline and following intervention. The estimated mean 24-hour urine output at baseline and during intervention combined was 933 (793–1,099) mL/day. The mean percentage FB$_1$ excreted in urine, based on FB$_1$ intake and the estimated mean daily urine output was 0.075%/day (0.054%/day to 0.104%/day).

**Discussion**

This is the first published study describing a quantitative correlation between a fumonisin biomarker and intake of this mycotoxin measured at the individual level. The investigation provides further validation following a previous report showing a positive correlation between the FB$_1$ urinary biomarker and different levels of maize-based tortilla consumption in a Mexican population (19). In addition, the current investigation applied an

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**Table 2.** The geometric means (95% confidence limits) of FB$_1$ levels in porridge and urine, as well as PDI in Centane, a rural area from the Eastern Cape Province of South Africa.

<table>
<thead>
<tr>
<th></th>
<th>FB$_1$ porridge, mg/kg</th>
<th>FB$_1$ PDI, µg/kg b.w./day</th>
<th>UFB$_1$, pg/mL</th>
<th>UFB$_1$C, pg/mg creatinine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td>0.89a (0.55–1.44)</td>
<td>4.84a (2.87–8.14)</td>
<td>225a (144–350)</td>
<td>470a (295–750)</td>
</tr>
<tr>
<td>Intervention</td>
<td>0.32b (0.27–0.37)</td>
<td>1.87b (1.40–2.51)</td>
<td>109b (85–138)</td>
<td>279a (202–386)</td>
</tr>
<tr>
<td>Reduction</td>
<td>64%</td>
<td>62%</td>
<td>52%</td>
<td>41%</td>
</tr>
</tbody>
</table>

$^a$PDI based on FB$_1$ levels in porridge (dry weight) consumed as assessed with 24-hour dietary recall questionnaires

$^b$UFB$_1$ was normalized with urinary creatinine

Means are significantly ($P < 0.05$) different within columns when followed by different letters

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**Figure 1.** The relationship between UFB$_1$C and FB$_1$ intake (natural log-transformed data) at baseline (left) and at baseline and intervention combined (right) is shown. The lines show the predicted linear regression for UFB$_1$C and the grey curves indicate the 95% confidence limits.
optimized sorting and washing method to maize, which reduced fumonisin exposure in a subsistence farming community as previously assessed by food analysis and food intake data (21). The biomarker was well correlated with FB1 exposure at the individual level and confirmed the efficacy of this simple and culturally acceptable intervention method.

Surveys previously conducted in subsistence farming communities in Africa have shown that mycotoxin contamination, such as fumonisin and aflatoxin, can be reduced by hand-sorting and/or washing of maize kernels (24, 25). Featured in this paper and our previous publication (21), the first intervention study to reduce fumonisin exposure by implementing these practices has been conducted recently in a rural subsistence-farming community in South Africa. The customary sorting and washing of maize kernels as food preparation procedures were optimized under laboratory-controlled conditions. The participants were trained to apply the simple 2-step method by identifying the infected maize kernels to ensure proper selection for removal and to follow the correct washing procedures. The intervention method is practical and culturally acceptable as it represents the optimization of existing local practices. The FB1 level in the maize-based porridge, prepared from the maize sorted and washed by the participants following training, was significantly ($P < 0.05$) reduced by 64% compared with the porridge prepared by the participants prior to training. The intervention study method effectively reduced the PDIs of FB1 by 62% (21).

The tortillas the participants consumed in the Mexican study were prepared from maize kernels which were boiled in alkaline water followed by prolonged steeping in fresh water (19, 26). Subsequently, the cooked kernels were washed and ground to yield masa from which the tortillas were prepared. The cooking of kernels in alkaline water partially converts the fumonisins to their hydrolyzed analogues, and therefore the tortillas contain both fumonisins and hydrolyzed fumonisins. The geometric mean UFB1C (134 pg/mg creatinine) in the high tortilla consumption group in the Mexican study (19) was more than 3-fold lower than at baseline in Centane (470 pg/mg creatinine). Based on the UFB1 level in the high tortilla consumption group, FB1 exposure was estimated to be 0.37 μg/kg body weight/day assuming a 1,500 mL/day urine output, a 60 kg body weight, and an assumption of a 1% excretion of ingested FB1 in urine, based on data in swine and nonhuman primates (27, 28). It is notable that in the current study the measured urinary excretion of FB1 was significantly lower than 1% (see the following text) and hence the intakes in Mexico may have been underestimated if urinary FB1 excretion in the 2 populations is similar. Re-estimating the PDI in the Mexican study, based on the estimated mean 24-hour urine output and the urine excretion rate estimated for this study, the FB1 PDI in Mexico (3.04 μg/kg body weight/day) was slightly lower than observed in Centane (4.83 μg/kg body weight/day). A recent study conducted in China with male and female participants reported median UFB1C levels of 390 and 3,910 pg/mg creatinine, respectively, in a high-risk hepatocellular carcinoma area (Fusui) and in a high-risk oesophageal cancer area (Huaian; ref. 18). The median level reported for Fusui was similar to Centane (451 pg/mg creatinine), whereas the median level in Huaian was more than 8-fold higher.

The mean UFB1 excretion in the Centane population, based on individual body weight, an estimated urine output, the actual FB1 content of the food consumed, and the food consumption (dry weight) was 0.075%. The correlation between FB1 intake and the urinary biomarker is influenced by the low proportion of FB1 excreted in urine and the interindividual variation of absorption and excretion of FB1 (19, 28, 29). This variation was observed in vervet monkeys where toxicokinetic studies revealed individual variations in urinary excretion with values of 0.25%, 0.66%, 1.0%, and 1.5% (28, 29). Experimental studies in rodents and swine reported urinary excretion of 0.4%–2% (27, 30). A more recent study in swine reported a 0.9% urinary excretion with peak urinary excretion between 8 and 24 hours following administration of a single oral dose of 5 mg FB1/kg (31). In a preliminary human study in the United States, participants consumed maize-based food that approximated maize consumption in urban Guatemala (32). The food was prepared from commercial masa and maize flour, containing 0.8 to 2.5 mg FB1/kg. The urinary excretion of less than 1% FB1 measured in the U.S. study was 10-fold higher than the less than 0.075% measured in this current study. It remains possible that there are ethnic differences or food matrix effects, which influence urinary FB1 excretion in human populations. Future studies to measure FB1 PDI in population would certainly be useful to improve our understanding on individual and ethnic variation in FB1 excretion through urine.

Due to the rapid urinary excretion rate of FB1, first-void urine was collected 12 hours following the last porridge meal consumed by the participants on 2 consecutive days at both baseline and intervention phases of the study. In a rat study following a single FB1 gavage dose (25 mg/kg body weight) UFB1 levels peaked at 12 hours, whereas levels declined rapidly, but FB1 was still detectable 10 days later (30). This study also suggested that UFB1 might reflect long-term exposure with chronic FB1 exposure at low levels in rats (1.0 mg/kg body weight/day). Therefore, residual FB1 from the previous days’ maize consumption in the current study could have contributed to the measured UFB1. Sources of FB1 other than maize could also have contributed to the individual exposure. However, as this subsistence community is reliant on maize almost to the exclusion of all other food commodities, the contribution of other sources is expected to be insignificant. This was verified by utilizing a validated questionnaire specifically designed for the local customary diet practices to obtain individual maize-based food consumption (33). The 52% reduction ($P < 0.05$) observed
in UFB₁ (41% in UFB₁(C) levels following intervention were comparable to the 62% reduction in FB₁ exposure as previously assessed by food analysis and food intake data (21). Indeed one of the advantages of assessing the intervention using a biomarker is that it provides a measure of the effect of the intervention on FB₁ exposure from all dietary sources, whereas, by definition, the maize and food analysis indicates only the effect on the target commodity itself.

Conclusion

The FB₁ urinary biomarker is quantitatively correlated with FB₁ exposure at the individual level. The biomarker permitted confirmation of the reduction in fumonisin exposure achieved with the culturally acceptable intervention method of hand sorting and washing maize. Utilization of this biomarker will improve assessment of fumonisin exposure and thus contribute to the assessment of the possible health impacts of fumonisin exposure as well as permitting evaluation of intervention strategies to reduce fumonisin exposure. Future intervention studies could be expanded to include larger numbers of both male and female participants including children. Significant advances in food safety and health in subsistence maize farming communities exposed to high levels of fumonisin could be possible by further development of these approaches.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Acknowledgments

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