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Short Communication

A comparison of 24 h urinary deoxynivalenol with recent *v.* average cereal consumption for UK adults

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Deoxynivalenol (DON) is a toxic fungal metabolite found on wheat, maize and barley. We previously reported a significant association between the amount of DON in a single 24 h urine sample and the average cereal intake over 7 d for 300 UK adults. In this more detailed analysis of the data, food diary information (*n* 255) for the day of urine collection (model I), the previous 24 h period (model II) and the day of urine collection plus the previous 24 h combined (model III) were further examined to assess whether the recent intake of cereal correlated more strongly with urinary DON, compared with the longer-term assessment of usual cereal intake from 7 d food diaries (model IV). DON was detected in 254/255 (99·6 %) urine samples (mean 12·0 μg/d; range not detected–66 μg/d). For all the models, total cereal intake was positively associated with urinary DON ($P<0·001$) in each model. The goodness of fit (adjusted R^2 value) was used to assess how well each model explained the variation in urinary DON. Model I provided a better goodness of fit (adjusted R^2 0·22) than model IV (adjusted R^2 0·19), whereas model III provided the best fit (adjusted R^2 0·27). These data suggest that the inter-individual variation in urinary DON was somewhat better explained by recent cereal intake compared with usual cereal intake assessed over 7 d.

Cereals: Deoxynivalenol: Mycotoxins: Urinary biomarkers

Deoxynivalenol (DON) is one of the most frequently observed *Fusarium* mycotoxins, and predominantly contaminates wheat, maize and barley[1]. DON has proven animal toxicity[2] and because of its stability during processing and cooking, human dietary exposure is frequent[1,3]. In ecological studies DON contamination of cereals has been linked to acute human toxicity[4,5]. Typical symptoms are similar to those in animals; they have rapid onset, including nausea, vomiting, abdominal pain, diarrhoea, headache, dizziness and fever[3]. In two well-documented incidents, DON contamination levels ranged between 0·3 and 92·8 mg/kg in China[4] and between 0·4 and 8·4 mg/kg in India[5]; thus intakes estimated at the low μg/kg body weight per d may be sufficient for poisoning. However, the lack of a biological exposure measure, or biomarker, for DON has severely restricted our ability to accurately assess exposure at the individual level; thus our understanding of a 'safe' level of exposure remains poorly defined.

In response we have developed a robust urinary measure for DON to assess exposure at the individual level[6]. This measure was applied to a survey of 300 UK adults to assess the frequency and range of detection of this putative biomarker in a single 24 h urine sample. The survey additionally assessed how well the typical wheat and maize (cereal) consumption over a 7 d period explained the variation in urinary DON[7]. Urinary DON was frequently detected and whilst the level was correlated ($P<0·0005$) with average cereal intake, it was notable that the variation in urinary DON was relatively poorly explained by self-reported cereal intake. In humans the toxicokinetics of DON has not been established. Urinary excretion of xenobiotics typically represents exposures over the previous 24–48 h time period. Thus the use of an average cereal intake over 7 d to assess the dietary contribution to urinary DON levels, collected over a single 24 h time period, may not be ideal. In this paper we report a more detailed analysis of the data presented by

Abbreviations: DON, deoxynivalenol; model I, food diary information for the day of urine collection; model II, food diary information for the previous 24 h period; model III, food diary information for the day of urine collection plus the previous 24 h combined; model IV, longer-term assessment of usual cereal intake from 7 d food diaries.

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Turner *et al.* [7], in which cereal intake during the day of urine collection and the previous 24 h were additionally evaluated.

Methods

Initial subject enrolment, including ethical approval and informed consent, and details of urinary DON assessment have been previously described in detail[7]. In brief, the UK adult National Diet and Nutrition Survey provided 7 d food diary information from 1724 individuals and a single 24 h urine sample, collected during the period of food diary recording[8]. The average cereal intake (g/d) was calculated based on major potential sources of dietary DON – bread (white, wholemeal, other bread), high-fibre and wholegrain breakfast cereal, sweet snacks (cakes, buns, biscuits), pasta, pizza, savoury snacks. The 'other bread' category included brown, high-fibre white, rye, gluten free, garlic bread, ciabatta, muffins, bagels, brioche, naan and paratha. The study population was divided into deciles of cereal intake and 100 subjects were randomly selected from each of the following cereal consumption groups: low (2nd/3rd decile), medium (5th/6th decile) and high (9th decile). Average cereal intake was then compared with a measure of 24 h urinary DON (μg/d). The urinary DON measure[6,7] involved immunoaffinity enrichment followed by LC-MS to quantify DON (ng/ml urine). The 24 h urine volume was recorded allowing conversion to μg DON/d.

In this further analysis, three additional models were constructed based on these pre-selected 300 individuals. Urinary DON was compared with cereal intake (a) on the day of urine collection (model I), (b) during the previous 24 h before the day of urine collection (model II) and (c) for the mean intake per d in the previous 24 h plus the day of urine collection combined (model III). Only 255 of the initial 300 individuals with urinary DON data had food diary information pertinent to all of these models. To provide a comparison with the original analysis, average 7 d cereal intake for these same 255 individuals was used (model IV). Beer was additionally included in models assessing the contribution of individual foods to urinary DON level, but was not included in models looking at total cereal intake due to the high water content of beer.

In this restricted subset only one urine sample was below the limit of detection (0·6 ng DON/ml urine). The mean DON level was 7·5 ng/ml urine (range from not detected to 56·4 ng/ml), or 12·0 μg/d (range from not detected to 66 μg/d). This latter measure was used in all statistical analysis. Measurements of urinary DON were natural log-transformed before statistical analysis. A multivariable regression analysis was conducted using Stata (version 10; Stata Corp. LP, College Station, TX, USA) to assess the contribution that total cereal intake, within each model, made to the level of urinary DON. The adjusted R^2 values were used to assess the proportion of the variability in urinary DON explained by the models. Subsequently the contribution that individual food items made to the level of urinary DON was tested. Each model included only the amount of a given food item being assessed, with adjustment for age, BMI and sex. The percentage increase in urinary DON per 50 g of food item was calculated from the correlation coefficients (and 95 % CI) obtained from multivariable regression models. Adjustments for both ethnic status (96·9 % white Caucasian) and vegetarian status (5·9 %) had little impact on the association between food intake and urinary DON.

Results

There was a similar frequency in the numbers of individuals that consumed at least one cereal item predicted to contribute to DON exposure: 236 (92·5 %), 238 (93·3 %), 253 (99·2 %) and 255 (100 %) for models I, II, III and IV, respectively. The mean for this cereal consumption was 175 (SD 130), 184 (SD 141), 180 (SD 108) and 181 (SD 80) g/d for models I, II, III and IV, respectively. For each model (I–IV) the amount of cereal consumed was divided into four categories: those with no intake of that food item and tertiles of the amount of intake. Kappa tests[9] were performed to assess the agreement between tertiles of amounts of cereal in each model against tertiles of the average amounts of cereal over the 7 d diary period. Kappa values were 0·26, 0·26 and 0·30 when comparing models I, II and III respectively with model IV, indicating only weak to fair agreement.

The associations between total cereal intake and urinary DON were assessed by regression analysis with adjustment for age, BMI and sex. For total cereal intake, adjusted R^2 values were 0·22, 0·16, 0·27 and 0·19 for models I, II, III and IV respectively, indicating that cereal intake on the day of urine collection better explained the variation (22 %) in 24 h urinary DON level compared with the average of 7 d (19 %). Model III provided a further improvement, with 27 % of the variation explained.

In a more detailed assessment, the associations between individual food groups and urinary DON within each of model III and model IV were compared (see Table 1). In both models the level of urinary DON increased with increased consumption of each of the food groups listed. In model III the amount of bread consumed was the strongest significant predictor of the level of urinary DON. Levels of DON increased by 27 % per 50 g increase in total bread (ratio 1·27 (95 % CI 1·18, 1·37); $P<0·001$). The rank order of the bread types in terms of their association with urinary DON level was wholemeal bread (ratio 1·27 (95 % CI 1·11, 1·45); $P<0·001$) > other bread (ratio 1·18 (95 % CI 1·06, 1·32); $P=0·003$) > white bread (ratio 1·11 (95 % CI 1·02, 1·21); $P=0·02$). Pasta and wholegrain/high-fibre breakfast cereal consumption was also strongly associated with urinary DON level. When comparing data from model III (recent intake) with model IV (average 7 d intake) the P values were more significant, and CI for the ratio estimates were narrower in model III, with the exception of sweet snacks. In addition, white bread and pizza were significantly associated with urinary DON in model III, but not in model IV.

Discussion

Cereals provide an important contribution towards a healthy diet. However, cereals are also frequently contaminated with toxic fungal metabolites[1]; thus it is important to understand the potential adverse contribution of such exposures to human health. Biomarkers of exposure offer an opportunity to examine exposures at the individual level, and provide useful data to inform epidemiological studies. We previously

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Table 1. Summary data of consumption of major cereal food items potentially contaminated with deoxynivalenol

	Model III (*n* 255)								Model IV (*n* 255)						
	Intake (g/d)		Consumers						Intake (g/d)		Consumers				
Food group	Mean	SD	*n*	%	Ratio	95 % CI	*P*	R^2	Mean	SD	*n*	%	Ratio	95 % CI	*P*	R^2
Pasta	28	58	67	26	1·15	1·06, 1·25	0·001	0·09	26	37	133	52	1·18	1·05, 1·35	0·008	0·07
Pizza	7	32	16	6	1·20	1·04, 1·39	0·015	0·07	10	23	62	24	1·07	0·87, 1·32	0·529	0·05
White bread	57	55	182	71	1·11	1·02, 1·21	0·020	0·07	61	46	235	92	1·02	0·92, 1·14	0·664	0·05
Wholemeal bread	18	35	67	26	1·27	1·11, 1·45	<0·001	0·09	17	31	98	38	1·31	1·13, 1·52	0·001	0·09
Other bread*	21	43	78	31	1·18	1·06, 1·32	0·003	0·08	19	32	127	50	1·20	1·04, 1·39	0·016	0·07
Wholegrain and high-fibre breakfast cereal	18	38	86	34	1·18	1·04, 1·34	0·009	0·07	19	32	126	49	1·27	1·09, 1·47	0·002	0·08
Sweet snacks	29	43	140	55	1·11	0·99, 1·24	0·072	0·06	29	31	209	82	1·22	1·05, 1·43	0·012	0·07
Savoury snacks	1	4	16	6	3·01	0·91, 9·93	0·072	0·06	1	3	36	14	1·68	0·25, 11·07	0·59	0·05
Beer	238	602	67	26	1·01	1·00, 1·01	0·155	0·05	223	436	112	44	1·01	1·00, 1·02	0·216	0·05
Total cereal	180	108	253	99	1·19	1·14, 1·23	<0·001	0·27	181	80	255	100	1·21	1·14, 1·28	<0·001	0·19

Model III, average daily consumption based upon previous 24 h and day of collection; model IV, average over 7 d.
*Brown, high-fibre white, rye, gluten-free, garlic bread, ciabatta, muffins, bagels, brioche, naan and paratha.

demonstrated that DON exposure was frequent in the UK and that the level of urinary DON was significantly associated with the average of recorded 7 d cereal intake[7]. In this more detailed analysis, cereal intake over a shorter and potentially more relevant time frame was determined in order to assess its contribution to the inter-individual variation in urinary DON. Kappa analysis indicated that despite similar frequency and intake of cereals occurring, intake patterns for the shorter time frame gave only a weak to fair correlation with the average intake over 7 d.

Regression analysis indicated that the variation in 24 h urinary DON levels was best explained in the model that assessed cereal intake on the day of collection and the previous day combined (model III) compared with the average of 7 d (model IV). This observation was consistent with our hypothesis that urinary DON will probably represent recent (previous 24–48 h) consumption of contaminated food items. When individual food items were considered, it was notable that consumption of both white bread and pizza were significantly associated with the level of urinary DON in model III but not model IV. DON contamination of wheat tends to be greatest in the bran fraction[10], and these data highlight the more significant contribution from wholemeal bread compared with any other food item. When individuals who consumed wholemeal bread (*n* 50) were removed from model III, subsequent regression analysis of this restricted dataset revealed a more significant correlation between the urinary measure and each of the remaining food groups (data not shown). Notably, the contribution of savoury snacks (ratio 5·10 (95 % CI 1·12, 23·34); $P=0·036$) and beer (ratio 1·01 (95 % CI 1·00, 1·02); $P=0·041$) were now significant.

Although small improvements in correlations with cereal intake were observed when considering different time frames, none of the models presented was able to explain the majority of the inter-individual variation in urinary DON. This observation may in part reflect the heterogeneous distribution of mycotoxins such as DON in food items, and is of itself persuasive of the need for an exposure biomarker. There are several additional potential explanations for these results. First, the self-reported cereal consumption data from the diaries will contain inaccuracies. Second, the kinetics of clearance of DON in humans is not understood and thus the timing of consumption of any contaminated food item may influence the measure of urinary DON. Assessment of the temporal variation of these measures, and indeed of DON intake itself against the urinary measure, may produce a better explanation of these relationships. Third, the present study focused on major food groups that could be readily assessed; other potential sources of exposure were not taken into account here.

It is also important to understand the relative contribution that a food item makes to DON exposure based on the actual amounts consumed. Whilst wholemeal bread consumption was the major predictor of urinary DON, the mean level of consumption was considerably lower than that for white bread. Thus for the study population as a whole, white bread provided the major source of DON. It is also important to emphasise that these data simply indicate exposure, not health risk. Our previous estimate of mean DON intake based on our biomarker data (0·3 μg/kg body weight per d)[7] was lower than those potentially linked to earlier poisoning

incidents[4,5]; however, we estimate that 5 % of UK adults may exceed the tolerable daily intake of 1 μg/kg body weight per d[11]. Within risk assessment it is always important to understand the variation in exposure within a population, not just the mean. We believe that the detailed understanding and use of this exposure measure for DON will strengthen epidemiological assessment of the potential risk from this toxin.

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The authors declare that there was no conflict of interest in the present study.

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