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A comparison of deoxynivalenol intake and urinary deoxynivalenol in UK adults

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Abstract

The relationship between deoxynivalenol (DON) intake and first morning urinary DON was examined in UK adults to validate the latter as a biomarker of human exposure. DON was assessed in first morning samples collected during a period of normal diet, a wheat-restriction intervention diet, and partial wheat-restriction intervention in which bread was allowed. During the partial intervention duplicate bread portions were collected for DON analysis. During the normal diet, partial intervention and full intervention, urinary DON was detected in 198/210 (geometric mean 10.1 ng DON mg^{-1} creatinine, 95% confidence interval (CI) 8.6–11.6 ng mg^{-1}; range nd–70.7 ng mg^{-1}), in 94/98 (5.9 ng mg^{-1}, 95% CI 4.8–7.0 ng mg^{-1}; range nd–28.4 ng mg^{-1}), and 17/40 (0.5 ng mg^{-1}, 95% CI 0.3–0.7 ng mg^{-1}; range nd–3.3 ng mg^{-1}) volunteers, respectively. A strong correlation between DON intake and the urinary biomarker was observed ($p < 0.001$, adjusted $r^2 = 0.83$) in models adjusting for age, sex and body mass index. These data demonstrate a quantitative correlation between DON exposure and urinary DON, and serve to validate the use of urinary DON as an exposure biomarker.

Keywords: *Biomarker; deoxynivalenol; diet; urine; validation; UK*

Introduction

Mycotoxins are toxic secondary metabolites produced by numerous species of fungi. Those of major concern for human health are the *Fusarium* toxins, including trichothecenes (deoxynivalenol (DON), nivalenol, T-2 toxin), the fumonisins and zearalenone, and the *Aspergillus* toxins, aflatoxins and ochratoxin A (OTA) (Miller 1995). DON is one of the most frequently occurring *Fusarium* mycotoxins and predominantly contaminates wheat, maize and barley in temperate regions (Canady et al. 2001, CAST 2003, SCOOP 2003). DON and similar trichothecenes bind to the 60S ribosomal subunit, which inhibits translation and activates a pathway known as the ribotoxic stress response (Pestka et al. 2004, Pestka & Smolinski 2005, Rotter et al. 1996). DON causes feed refusal, decreased weight gain, gastroenteritis, cardiotoxicity and teratogenicity in animals (Rotter et al. 1996, Pestka & Smolinski 2005). In many species acute DON toxicity elicits an emetic response (Rotter et al. 1996) which has given rise to the alternative name 'vomitoxin'. Consumption of DON-contaminated cereals has been associated with numerous poisoning incidents in China between 1961 and 1991, at times affecting thousands of individuals (Luo 1994, Pestka & Smolinski 2005). In these outbreaks typical symptoms were similar to those observed in animals, notably being characterized by a rapid onset, nausea, vomiting, abdominal pain, diarrhoea, headache, dizziness and fever. In one well-documented incident DON contamination of wheat ranged between 0.3 and 92.8 mg DON kg^{-1} (reviewed by Pestka & Smolinski 2005), data suggesting that acute toxicity may occur at exposures estimated in the low μg kg^{-1} bw daily range. A major incident of food poisoning also occurred in the Kashmir valley, India affecting approximately 50 000 individuals with DON levels between 0.4 and 8.4 mg kg^{-1} (Bhat et al. 1989).

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DON also modulates immune function *in vitro* (Gray & Pestka 2007, Pestka et al. 2004, Meky et al. 2001, Van de Walle et al. 2008), including suppression of the immune response to pathogens, possibly through restricted nitric oxide production in macrophages (Sugiyama et al. 2010) and/or alterations in macrophage receptors (Wache et al. 2009). *In vivo* DON impairs growth at exposure levels below those that restrict appetite (Amuzie & Pestka 2010, Pestka & Smolinski 2005), an effect recently suggested to be linked to growth hormone signalling and insulin-like growth factor suppression (Amuzie et al. 2010, Voss 2009).

Given the predicted common dietary exposure to DON, it is important to understand the potential health risks, particularly from chronic exposures. One of the major challenges for epidemiology is exposure assessment. As with many other mycotoxins, heterogeneous DON contamination of food items, e.g. different batches or manufacturers of white bread, makes exposure assessment based on dietary analysis problematic. Biological measures (or biomarkers) potentially offer improved exposure assessment. A urinary assay for DON, first reported by Meky and colleagues (2003) using immunoaffinity enrichment and liquid chromatography–mass spectrometry (LC-MS) was refined by inclusion of $^{13}C_{15}$-DON as an internal standard (Turner et al. 2008a, b). DON was frequently detected in urine from both UK (Turner et al. 2008c) and French (Turner et al. 2010) adults, but despite a positive correlation being observed between the urinary measure and cereal intake (Turner et al. 2008b, c), the interindividual variation in urinary DON was still relatively poorly explained by cereal intake.

The current study aimed to validate further the urinary DON biomarker by better defining the relationship with DON exposure. The design involved repeat measures of DON in urine during a period of normal diet consumption and an intervention period where major potential sources of DON in the diet were either avoided ('full intervention'), or restricted to bread only ('partial intervention'), for 4 days. For the latter group duplicate portions of bread were collected throughout the partial intervention for DON analysis such that the quantitative relationship between the measure of DON intake and urinary DON could be assessed.

Material and methods

Study design

Thirty-five volunteers from the University of Leeds, UK, 17 male, 18 female, aged 21–59 years were recruited to take part in the biomarker validation study. Height and weight were self-reported to obtain individuals' body mass index (BMI). First morning urines were collected from each individual over 12 days from Monday (day 1) through to the second Friday (day 12). No urine was collected on the Saturday or Sunday, thus urinary samples were available on days 1–5 and 8–12. Urine was stored frozen at -20°C prior to analysis. A detailed semi-weighed food diary was completed by each volunteer to assess the amounts and types of foods consumed on each day prior to urine collection, and in particular the type and amount of cereals consumed. Foods were either weighed where possible or serving size described using pack weights and standard units, e.g. tablespoons or slices. Collection of dietary information commenced on the Sunday (day 0) and ran until the second Thursday (day 11). Thus for each day the urine sample was paired with the dietary information from the previous day. From days 0 to 7 ($n=8$ days) all individuals consumed their normal diet. On days 8–11 ($n=4$ days) adherence to a restricted 'intervention' diet was requested. For 25 individuals a partial intervention was undertaken in which bread was allowed but volunteers were requested to refrain from consumption of other foods likely to be contaminated with DON (pasta, pizza, sweet snacks (biscuits, cakes, buns, pastries)), breakfast cereals (excluding oat and rice cereals), flour-, wheat- or maize-based savoury snacks). A portion of each bread item consumed during the partial intervention on each day was obtained in order to measure DON contamination. The level of DON contamination and the amount of bread consumed allow the daily DON intake to be assessed. A further ten individuals undertook a full intervention in which they were requested to restrict all possible major sources of DON. A clear guideline of all foods to avoid was provided to both intervention groups, alongside suggested alternative sources of carbohydrate, e.g. potatoes, rice. Informed consent was obtained from all volunteers prior to the study and ethical approval was obtained from the Leeds Teaching Hospitals NHS Trust Research Ethics Committee.

Food diaries

Food diaries were fully coded using an in-house Microsoft Access-based dietary analysis package, which uses the UK Composition of Foods (Holland et al. 1992). To assess compliance with the intervention, the frequency of consumption of specific food groups, i.e. bread, pasta, pizza, sweet snacks (biscuits, cakes, buns, pastries), breakfast cereals (excluding oat and rice cereals), flour-, wheat- or maize-based savoury snacks during normal diet was compared with that during the intervention. In addition the weights of these specific food items were estimated and the intake of cereal-based foods (specifically wheat and maize) was determined for each individual. Beer represents a potential source of DON exposure; consumption levels were identified where individual food items were compared with urinary DON, but was not included as part of the main 'cereal group' for statistical analysis due

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to its large water content. Other potential sources of DON do occur in the diet, such as in sauces and thickeners. However, their contribution to DON intake was estimated to be of relatively minor importance.

Measurement of urinary DON

Total urinary DON was measured in 1 ml of urine essentially using the method of Turner and colleagues (2008a, 2010). In brief, each urine sample was defrosted at room temperature, centrifuged at 2000*g* (15 min, 4°C) and the supernatant adjusted to pH 6.8. A 1 ml sample was removed and mixed with 20 ng $^{13}C_{15}$ DON (Biopure Referenzsubstanzen, Tulln, Austria) as an internal standard (IS). The mix was incubated with 7000 units of β-glucuronidase (Type IX-A from *Escherichia coli*; Sigma, Poole, Dorset, UK) overnight at 37°C with continuous gentle shaking. Following digestion, the samples were centrifuged (2000*g*; 15 min; 4°C) and the supernatant diluted to 4 ml with phosphate-buffered saline (PBS) (pH 7.2). The diluted material was passed through wide-bore DON immunoaffinity columns (Vicam, Watertown, MA, USA) to extract the DON as per the manufacturer's instructions. DON was eluted from columns with 4 ml of methanol and the extracts dried *in vacuo* using a Savant Speed Vac and reconstituted in 250 µl of 10% (v/v) ethanol for LC-MS analysis. Two aliquots each of a blank urine spiked with 10 ng ml^{-1} DON (QC-10) and a PBS blank were also spiked with the internal standard ($^{13}C_{15}$ DON) as above and extracted alongside each batch of test samples as quality controls. LC-MS was conducted according Turner et al. (2008a). The LC-MS standard curve was constructed using seven concentrations of DON, range 2–250 ng ml^{-1} in 10% v/v ethanol, each spiked with the IS, and a blank containing 10% v/v ethanol spiked with the IS. Selective ion recording was used to quantify the individual DON levels by reference to the IS. Test samples were quantified by reference to a response-ratio calibration curve generated by Quanlynx software. The detection limit for the assay was 0.5 ng DON ml^{-1} of urine. All samples were analysed in batches of 20 with two QC-10 and two PBS blanks. The PBS blanks gave rise to a signal corresponding to 0.8 ng ml^{-1}; CV% of 42 samples was 5.8. The mean value of the two PBS samples within each run was subtracted from both the QCs and unknowns tested in that run. For the QC the overall mean level of DON was 10.2 ng ml^{-1}, CV% 8.3, $n = 42$. The signal for the PBS blank may arise in part from the IS ($^{13}C_{15}$-DON) which contains a small percentage of DON.

Creatinine analysis

Urinary creatinine analysis was conducted for all samples using an in-house microtitre plate assay modified from the alkaline-picrate method (Varley 1967). Urinary creatinine (mg ml^{-1} urine) was used to adjust the DON concentration from ng ml^{-1} urine to ng mg^{-1} creatinine.

Analysis of DON in bread

A total of 118 bread samples were obtained during the 4-day partial intervention. Some individuals supplied multiple bread slices from the same loaf and thus a number of replicate bread samples were identified from food diaries. A total of 82 distinct samples were analysed for DON by RHM Technology (Premier Foods, UK). The laboratories are accredited to UKAS 17025 standards. The method of analysis involved solvent extraction and gas chromatography (GC)-MS analysis. The detection limit was 5 µg kg^{-1}. The mean recovery was 96% with a range of 85–103%, and was within the 70–110% acceptable limits (EU Regulation 2006).

Statistical analysis

Any urinary sample with non-detectable levels of DON was assigned a value of half the limit of detection, i.e. 0.25 ng ml^{-1}. Urinary DON data were natural log-transformed prior to statistical analysis. Following statistical analysis all mean urinary DON values quoted in the text are geometric means. Demographic and dietary comparisons were conducted using the unpaired *t*-test. Regression analysis was used to compare cereal or DON intake with urinary DON. Multivariate (MV) models presented included adjustment for age, sex and BMI. All data analysis was conducted using STATA version 9.0 (STATA Corp., TX, USA).

Results

Demographics

Among the 35 subjects (17 male, 18 female) in the study the mean age was 37 years (range 20–59) and the mean BMI was 24.6 (range 20.2–34.0) with no difference in these parameters between men and women. The subjects were all white Caucasian except for one from south-east Asia.

Food diaries

Food diaries were kept for 12 days by 30 individuals, for 11 days by three individuals and for 10 days by two individuals; thus a total of 413 days of diary information was recorded. Maintenance of basic macronutrient consumption throughout the duration of the study was assessed during the normal diet and the intervention diet. During the normal diet the mean daily consumption of

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protein, fat, carbohydrate and total energy was 89 g daily (95% confidence interval (CI) 82–95), 88 g daily (95% CI 82–93), 267 g daily (95% CI 247–286) and 9442 KJ daily (95% CI 8865–10019), respectively. These intakes are in line with estimated average requirements for health (Department of Health 1991). Energy and macronutrient intakes slightly decreased during the intervention phases, although decreases were only statistically significant for protein (-10.1%, 95% CI -16.1 to -4.8, $p = 0.02$) and energy (-7.9%, 95% CI -13.8 to -2.1, $p = 0.01$) during the partial intervention; no significant decreases were observed during the full intervention phase.

The amounts and frequency of cereal and bread consumption are presented in Table 1. During consumption of the normal diet (over 8 days) cereal was consumed on at least 5 days with an average consumption per person of 206 g daily (range 0–658 g daily). Bread contributed on average approximately half of the total cereal intake during consumption of the normal diet.

During the intervention wheat- or maize-based cereal was consumed by 28 of 35 individuals on at least one occasion but with only three of these individuals coming from the full intervention group. Nine subjects in the partial intervention consumed cereals other than bread; thus compliance with the suggested diet was good but not absolute. Nevertheless, overall, there were 122 of 138 (88.4%) person days where compliance with the suggested diet was maintained; and the mean consumption of cereal per person per day was around 30-fold lower in the full intervention group (mean 7 g daily, range 0–150 g daily) than in the normal diet period (mean 206 g daily, range 0–658 g daily).

Table 1 Frequency and amount of cereal/bread consumed by 35 volunteers.

	Normal diet	Partial intervention	Full intervention
	8 days	4 days	4 days
Cereal consumption			
Number of consumers/ total[a]	35/35	25/25	3/10
Mean days per person[b]	8 (5–8)	4 (2–4)	1 (0–4)
Number of days/total days[c]	264/271	93/99	6/39
Mean (range) (g daily[d])	206 (0–658)	159 (0–455)	7 (0–150)
Bread consumption			
Number of consumers/ total[a]	35/35	25/25	0/10
Mean days per person[b]	6 (1–8)	4 (2–4)	0
Number of days/total days[c]	213/271	92/99	0/39
Mean (range) (g daily[d])	110 (0–535)	155 (0–455)	0

[a]On at least 1 day. [b]Mean (range) of the number of days each person consumed the food item. [c]Total number of person days when consumption occurred. [d]Mean (range) amount of food item consumed per day.

During the partial intervention all individuals consumed cereal (bread and other sources) on at least two occasions and the majority consumed cereal on all 4 days. The mean cereal consumption per person was 159 g daily (range 0–455 g daily) and as required in the protocol the overwhelming contribution to total cereal consumption came from bread (mean 155 g daily, range 0–455 g daily). Eight individuals consumed non-bread sources of cereals on one occasion and one individual did so on 2 days. Overall the mean consumption of non-bread cereal was modest (mean 5 g daily, range 0–125 g daily). The consumption of pasta was the largest single non-bread food item consumed on any day (108 g).

Urinary DON

During the normal diet phase of the study 35 individuals provide six urine samples each (total $n = 210$). DON was detected in 198/210 (94.2%) of these samples; overall mean 11.6 ng DON ml^{-1} urine (range nd–78.2 ng ml^{-1}). For the partial intervention, 23 individuals gave four samples and two individuals gave three samples each (total $n = 98$). DON was detected in 94/98 (95.9%) of these samples; overall mean 6.3 ng ml^{-1} (range nd–34.0 ng ml^{-1}). For the full intervention, ten individuals gave four samples each. DON was detected in 17/40 (42.5%) of the samples; overall mean 0.7 ng ml^{-1} (range nd–3.2 ng ml^{-1}) (Table 2).

All urinary DON data were also adjusted for creatinine. During the normal diet phase of the study the overall mean was 10.1 ng DON mg^{-1} creatinine (range nd–70.7 ng mg^{-1}). For the partial intervention the overall mean was 5.9 ng mg^{-1} (range nd–28.4 ng mg^{-1}), and for the full intervention it was 0.5 ng mg^{-1} (range nd–3.3 ng mg^{-1}) (Figure 1 and Table 2). Within 24 h of the full intervention commencing the average reduction in urinary DON (ng mg^{-1} creatinine) was 83% of that measured during the normal diet. There was no significant difference in mean urinary DON by day within any given dietary period of the study, i.e. normal diet, partial or full interventions. The overall mean urinary DON level within each phase

Table 2. Comparison of cereal intake and urinary deoxynivalenol (DON) by phase of study.

	Normal diet	Partial intervention	Full intervention
Mean cereal intake, g daily (%)[a]	206	159* (77)	7** (3)
Mean urinary DON ng ml^{-1} (%)[a]	11.6	6.3* (54)	0.7** (3)
Mean urinary DON ng mg^{-1} creatinine (%)[a]	10.1	5.9* (58)	0.5** (3)

[a]Percentage of normal diet. *Mean value statistically different from normal diet ($p < 0.001$). **Mean value statistically different from both normal diet and partial intervention ($p < 0.001$ for both).

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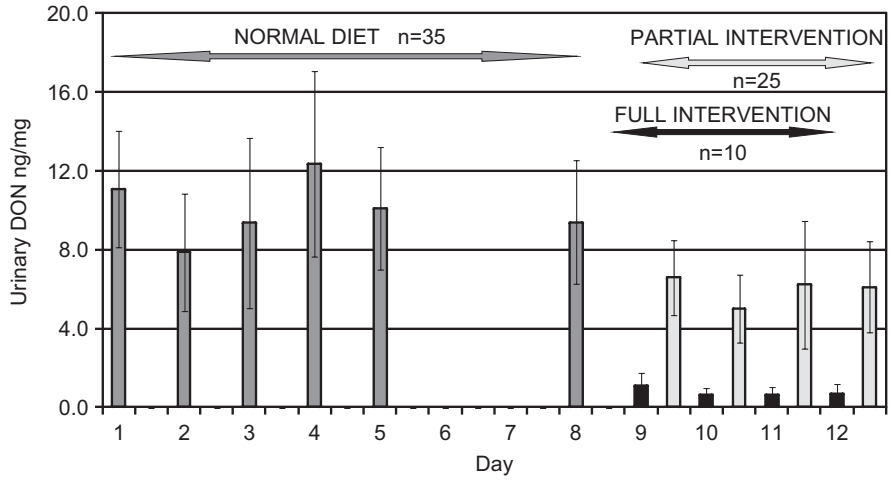

Figure 1. Summary of urinary deoxynivalenol (DON) (ng mg^{-1}) by day of study. The day 1 urine was collected on the first Monday and the day 12 urine was collected on the following Friday. No urine samples were collected for day 6 (Saturday) or day 7 (Sunday). Dietary activity refers to the consumption on the day prior to urine collection. Food diaries were maintained throughout the study, including days where no corresponding urine sample was collected. Urinary DON data for days 1–5 and day 8 (dark grey, $n = 35$) represent DON levels following the consumption of the 'normal' diet. Urinary DON data for days 9–12 (light grey, $n = 25$) represent DON levels following the consumption of a cereal restricted diet in which bread was the only major source of DON: 'partial intervention'. Urinary DON data for days 9–12 (black, $n = 10$) represent DON levels following the consumption of a cereal-restricted diet in which no major source of DON were permitted: 'full intervention'. For each day urinary DON levels are presented as a mean and 95% confidence intervals.

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of the study reflects the mean cereal intake within that phase (Table 2).

Cereal intake and urinary DON during the normal diet

Female subjects had higher (albeit not significant, $p > 0.25$) levels of urinary DON (mean 6.1 ng mg^{-1}; 95% CI 4.6–7.3) compared with male subjects (mean 5.8 ng mg^{-1}; 95% CI 4.6–7.3). Neither cereal nor bread consumption were significantly associated with sex or other demographic measures ($p > 0.25$ for all). In MV regression analysis (adjusting for age, sex and BMI) daily cereal consumption was significantly ($p < 0.001$; adjusted $R^2 = 0.230$) associated with the subsequent day's urinary DON level. In multivariate regression analysis (assessing the contribution of individual food items during the normal diet with urinary DON) bread ($p < 0.001$; adjusted $R^2 = 0.195$), sweet snacks ($p = 0.016$; adjusted $R^2 = 0.091$), pizza ($p = 0.019$; adjusted $R^2 = 0.090$) and beer ($p = 0.008$; adjusted $R^2 = 0.025$) were significantly associated with urinary DON.

DON contamination of bread

DON was detected in all 82 (100%) bread samples; mean 74 μg kg^{-1}; range 20–316 μg kg^{-1}. Bread was classified as white, seeded, white with added fibre (W-fib), granary/wholemeal (Gr/Whl) or other. Samples classified as Gr/Whl had significantly ($p < 0.001$) higher mean DON levels (89 μg kg^{-1}; 95% CI 78–101, $n = 33$) compared with those classified as white, W-fib and seeded combined (56 μg kg^{-1}; 95% CI 47–68). The contamination level and the amounts of bread consumed were used to assess DON intake. The average daily DON intake was 10.6 μg daily (range 0–42.5 μg daily).

DON intake and urinary DON level

Scatter-plots of the previous days DON intake from bread (partial intervention) against urinary DON (days 9–12) are presented in Figure 2 for each day of urine collection (unadjusted $R^2 = 0.56$, 0.49, 0.54. 0.64, respectively; $p < 0.001$ for all), and then as a mean of the four days measures (unadjusted $R^2 = 0.74$). For MV models adjusting for age, sex and BMI, urinary DON was significantly positively ($p < 0.0001$ for all) associated with DON intake on each of the 4 days of comparison (adjusted $R^2 = 0.63$, 0.68, 0.65, 0.63 and 0.83, respectively). Thus DON intake provided a good correlation with urinary DON on a daily basis, but the correlation was even stronger, explaining 83% of the variance when average intake of DON over days 8–11 and average urinary DON (days 9–12) were used.

The current recommended tolerable daily intake (TDI) for DON is 1000 ng kg^{-1} bw daily (SCF 2002). During the partial intervention phase we have an accurate measure of DON intake based on bread consumption. No individuals exceeded the TDI during the bread only phase (maximum intake was 582 ng kg^{-1} bw daily). The 24-h urinary excretion of DON was estimated by calculating creatinine clearance as a

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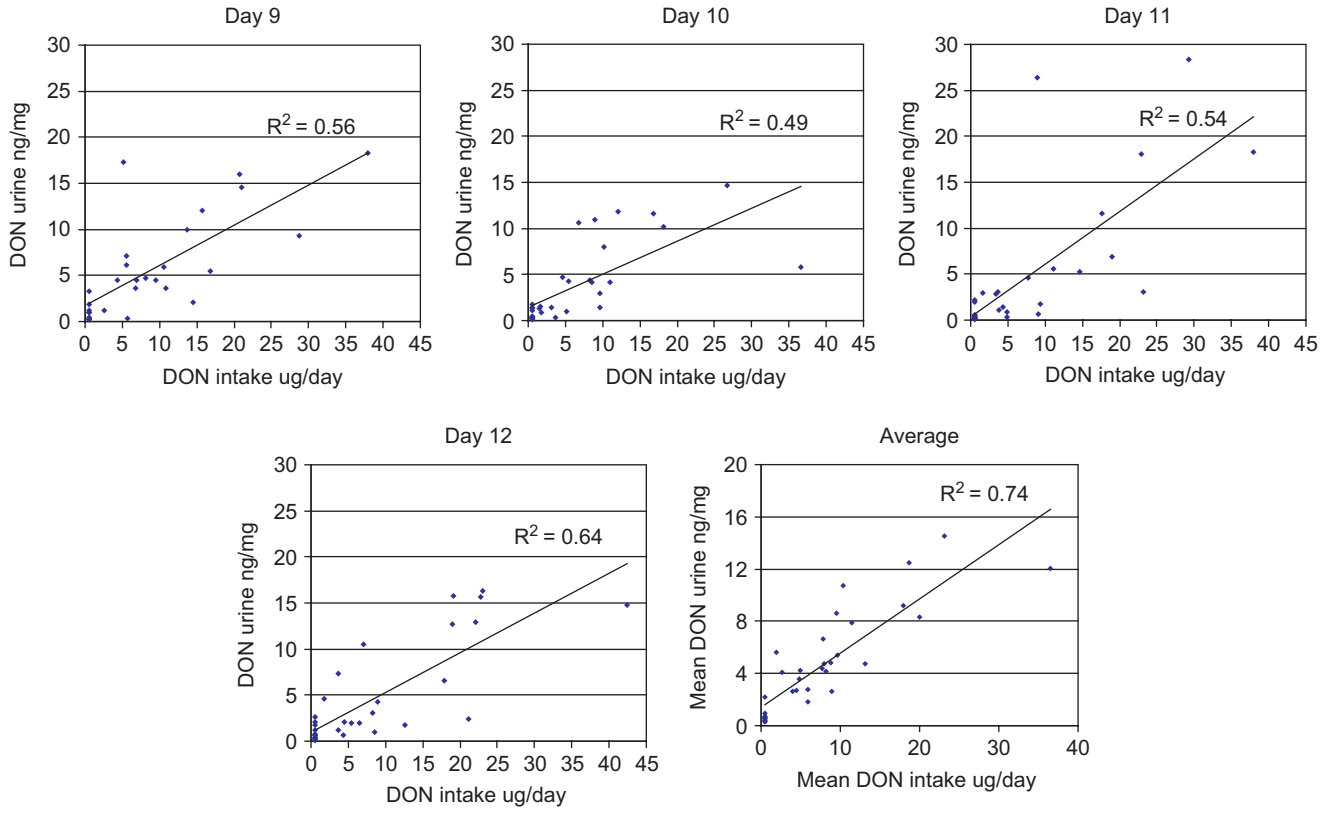

Figure 2. Scatter-plot of urinary deoxynivalenol (DON) against DON intake. Scatter-plots for each day of urine collection (day 9 to day 12) for the intervention phase of urinary DON against the DON intake on the day of urine sample, and a scatter-plot of the average urinary DON against average DON intake during this period. Unadjusted R^2 were 0.56, 0.49, 0.54, 0.64, 0.74, respectively.

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function of body mass, age and sex (Clinical calculator; http://www.clinicalculator.com/english/nephrology/excrea/excrea.htm). The daily urinary creatinine clearance was used to estimate the daily urinary DON excreted using the DON concentration in ng DON mg^{-1} creatinine. For those on the partial intervention the average DON intake per day (calculated from the bread intake recorded in food diaries and the DON contamination level of duplicate bread samples) for each person, and the average urinary DON excreted per day, were used to calculate the average percentage excretion of DON via the urine. The overall average excretion per day was 72.3% (95% CI 59.1–85.5%) and was not associated with sex, age, weight or DON intake (p >0.4 in all comparisons).

Using this estimated mean excretion per day and the individual levels of urinary DON (ng DON mg^{-1} creatinine), estimates of DON intake were then made for all individuals during consumption of the normal diet (Table 3). The overall estimated mean intake was 298 ng kg^{-1} bw daily (95% CI 218–362). The estimated intake exceeded the recommended TDI for 6/35 (17%) of individuals on one or more occasion, and a further 13 individuals had one or more days when estimated intake exceeded 50% of the TDI.

Discussion

Mycotoxins occur frequently in cereals with an estimated 25% of agricultural commodities contaminated worldwide. Due to the ubiquitous DON contamination of cereals in temperate regions of the world, and their resistance to degradation during processing, DON exposure in the UK will be common (SCOOP 2003). For most mycotoxins an understanding of the health consequences of exposure is hampered by poor exposure assessment but biomarkers may improve this situation. The most frequently analysed biological matrices are serum (or plasma), blood cells, urine, saliva or faeces. The toxicokinetics of DON in swine has been examined (Prelusky et al. 1988, Goyarts & Danicke 2006). Following DON consumption a rapid but transient high level of DON occurs in serum. Extrapolation of these data to estimated DON exposures in humans would suggest that serum may only be useful to examine episodes of acute exposure to high levels. As far as we are aware there are no published reports on the transfer of DON to saliva. Faecal excretion of DON represents approximately 2–5% of intake in swine (Goyarts & Danicke 2006) and 10% in rats (Meky et al. 2003). However, faeces are not typically collected in epidemiological studies, and

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Table 3. Summary of estimated deoxynivalenol (DON) intake during the normal diet.

Sample	Sex	Estimated DON (ng kg^{-1} bw daily), mean (range)	Frequency (500 ng kg^{-1} bw daily exceeded)	Frequency (1000 ng kg^{-1} bw daily exceeded)
1	M	235 (79–325)	0/6	0/6
2	F	104 (8–242)	0/6	0/6
3	F	336 (54–1159)	1/6	1/6
4	F	240 (76–570)	1/6	0/6
5	M	304 (89–572)	1/6	0/6
6	F	80 (23–164)	0/6	0/6
7	M	423 (130–967)	1/6	0/6
8	F	670 (297–1208)	4/6	1/6
9	F	316 (107–637)	1/6	0/6
10	M	91 (37–148)	0/6	0/6
11	F	75 (7–199)	0/6	0/6
12	F	179 (70–255)	0/6	0/6
13	F	1046 (151–1747)	5/6	3/6
14	M	31 (9–68)	0/6	0/6
15	M	315 (198–505)	1/6	0/6
16	M	277 (179–355)	0/6	0/6
17	M	255 (9–1168)	2/6	1/6
18	M	142 (76–252)	0/6	0/6
19	F	213 (48–474)	0/6	0/6
20	M	362 (55–794)	2/6	0/6
21	M	318 (132–498)	0/6	0/6
22	F	168 (43–664)	1/6	0/6
23	F	490 (169–904)	3/6	0/6
24	M	261 (67–443)	0/6	0/6
25	F	313 (198–568)	1/6	0/6
26	M	144 (8–319)	0/6	0/6
27	M	334 (149–702)	1/6	0/6
28	F	421 (276–636)	2/6	0/6
29	F	276 (98–552)	1/6	0/6
30	M	878 (538–1244)	4/6	2/6
31	F	250 (101–636)	1/6	0/6
32	F	9 (7–18)	0/6	0/6
33	M	321 (84–1189)	1/6	1/6
34	M	164 (30–326)	0/6	0/6
35	F	127 (7–346)	0/6	0/6

The estimated DON intake was calculated from the DON concentration measured in urine samples by taking into account the overall average DON excretion per day of 72.3%.

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do not represent an ideal matrix for exposure assessment. In contrast, urine is commonly collected and animal models indicate that >30% of DON will be transferred to urine in both rats and swine (Meky et al. 2003, Goyarts & Danicke 2006), thus urinary DON provides a potential candidate for an exposure biomarker.

We previously demonstrated the utility of a highly robust urinary assay to measure DON (Turner et al. 2008a), and have applied this to a set of urine samples from the UK adult National Diet and Nutrition Survey stratified for cereal consumption (Turner et al. 2008c). Urinary DON was significantly associated with cereal consumption (p <0.0001); however the variation in urinary DON in a single 24-h urine was relatively poorly explained by the variation in either average cereal intake over 7 days, adjusted $R^2 = 0.23$, $n = 300$, or cereal intake in the previous 24 h plus the day of sampling, adjusted $R^2 = 0.27$, $n = 255$ (Turner et al. 2009). In the present study a similar positive correlation was observed between cereal consumption and the urinary measure, but again the variation in urinary DON remained poorly explained by this parameter ($R^2 = 0.23$). The heterogeneous contamination of wheat and maize food items with DON will likely be the major reason for this apparent moderate association between cereal intake and the urinary measure. Reporting errors when obtaining food diary information may additionally contribute.

One of the critical steps in the validation of an exposure biomarker is demonstrating a clear dose–response relationship with the exposure, in this case the amount

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of DON ingested. The current study assessed individual levels of DON intake over 4 days using a duplicate food sampling and food diary approach, in which bread was the only major potential source of DON exposure. Our previous data revealed that within the UK, bread was consumed more frequently and at higher levels than any other food items at risk for contamination by DON (Turner et al. 2008a, c, 2009); and was the food item most strongly associated with urinary DON levels. Thus a focus on bread as the sole source of DON in a cereal-restricted diet provided an opportunity for a quantitative comparison of DON intake with the urinary biomarker. Bread consumption during the partial intervention was frequent and analysis of the collected portions of bread samples revealed considerable variation in the levels of DON contamination. High-fibre, granary and wholemeal breads had significantly higher levels of DON compared with other breads, an observation in line with the predicted accumulation of DON in the bran fraction of wheat (JECFA 2001).

During this partial intervention phase, DON intake on each of the previous days was significantly (p <0.001) associated with the urinary measure on the subsequent day. The variation in urinary DON was well explained in MV models for each day (adjusted R^2 = 0.63, 0.68, 0.65 and 0.63, respectively). Furthermore, the correlation between the average DON intake over the 4 days and the average urinary DON level was even stronger (adjusted R^2 = 0.83). Similar exposure biomarker validation approaches have been conducted for two *Aspergillus* mycotoxins, the liver carcinogen aflatoxins B1 (Groopman et al. 1993, 2002) and the nephrotoxin, OTA (Gilbert et al. 2001). For the *Fusarium* mycotoxins, fumonisins, the ratio of sphinganine and sphingosine in biofluids has been suggested as a possible measure of fumonisins exposure (Turner et al. 1999, Nikiema et al. 2008, Shephard et al. 2007), and urinary fumonisin B1 (FB1) has been shown to be correlated with consumption of maize-based tortillas (Gong et al. 2008), but no quantitative comparison with FB1 intake has yet been reported.

Using the DON intake and urinary biomarker data from the intervention phase of the study, the mean transfer of DON to urine was estimated at 72.3% (95% CI 59.1–85.5). This value was higher than that reported for swine (49.7%) dosed at 163 μg kg^{-1} bw daily, although not dissimilar to the value of 61.8% in the control animals with a 40-fold lower DON exposure resulting from the natural level of DON contamination in the swine feed (Goyarts & Danicke 2006). The intake in the swine control animals was close to the upper end of exposures predicted to naturally occur in humans (SCOOP 2003, Turner et al. 2008b).

Based on the estimated mean transfer of DON to urine, the estimated mean DON intake for individuals during the consumption of their normal diet was 298 ng kg^{-1} bw daily (95% CI 218–362). The estimated intake exceeded the recommended TDI (SCF 2002) for 6 of 35 (17%) of individuals on one or more day, and a further 13 individuals had one or more days when the estimated intake exceeded 50% of the recommended TDI. Previously rough estimates of DON intake, based on the urinary levels of DON were reported for the UK adult NDNS (Turner et al. 2008c). Here based on the additional precision about percentage transfer of DON into urine in people, we refine that mean estimate from 319 ng kg^{-1} bw daily to 222 ng kg^{-1} bw daily with 2/300 individuals predicted to exceed the recommended TDI. However, it should be noted that the latter individuals were a stratified sample based on cereal consumption and no samples were taken from individuals in the highest decile of cereal intake in the NDNS survey.

One caveat in our estimates of DON intake is that spot or first morning void urine samples are not necessarily ideal for assessment of daily exposures. Many studies have documented that creatinine-adjusted analyte concentrations serve as a good surrogate for size-related dose (reviewed by Barr et al. 2005). In our survey all urinary DON data were adjusted for creatinine for this reason. In practical terms morning urines are more applicable to epidemiological studies and from the data presented here it appears that urinary DON in such samples strongly reflects the previous days DON intake. Another caveat is that we only considered wheat and maize as sources of DON from the food diaries. It is likely that these represent the major sources in the diet; nevertheless other sources such as soups and sauces may also contribute but were not analysed in this survey.

In conclusion, this study demonstrates a strong quantitative correlation between DON intake and a putative exposure biomarker, first described by Meky et al. (2003). Previous experience with aflatoxin biomarkers has demonstrated how valuable such tools can be to understand exposure, in evaluating the contribution of mycotoxins to human health effects (Gong et al. 2002, IARC 2002, Jiang et al. 2005, Turner et al. 2003, 2007), and the effectiveness of interventions to reduce exposure (Egner et al. 2001, Kensler et al. 1998, Turner et al. 2005, Wang et al. 2008). It is now imperative to incorporate this DON exposure biomarker into a well-designed epidemiological investigation to assess the potential health effects of exposure. Such studies may also incorporate metabonomic and/or proteomic outcomes measures to assist in identifying the mechanisms by which DON may exert adverse health effects in humans (Hopton et al. 2010).

Declaration of interest

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