Biomonitoring of concurrent mycotoxin exposure among adults in Sweden through urinary multi-biomarker analysis

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Abstract
Mycotoxin producing moulds may contaminate numerous agricultural commodities either before harvest or during storage. A varied diet consisting of different foods may therefore be contaminated with a range of mycotoxins. The aim of the present study was to study concurrent exposure to mycotoxins through urinary multi-biomarker analysis, as well as its possible associations with the diet.

Urinary samples from 252 adults, participating in the Swedish national dietary survey Riksmaten 2010-11, were collected together with a 4-day diet record. Concurrent mycotoxin exposure was studied using a multi-biomarker LC-MS/MS method. The results revealed that exposure to mycotoxins is common and concurrent exposure to more than one toxin was found in 69% of the study population. However, when comparing the number of toxins detected with the reported consumption data it was difficult to distinguish food patterns which would indicate an increased risk of exposure to many mycotoxins simultaneously.

This is the first study to investigate concurrent mycotoxin exposure and urinary levels of fumonisin B_1 (FB_1), fumonisin B_2 (FB_2), nivalenol (NIV), ochratoxin A (OTA), zearalenone

Abbreviations: AFB_1, aflatoxin B_1; AFB_2, aflatoxin B_2; AFG_1, aflatoxin G_1; AFG_2, aflatoxin G_2; AFM_1, aflatoxin M1; DON, deoxynivalenol; DOM-1, de-epoxydeoxynivalenol; FB_1, fumonisin B_1; FB_2, fumonisin B_2; NIV, nivalenol; OTA, ochratoxin A; ZEA, zearalenone; α-ZOL, α-zearalenol; β-ZOL, β -zearalenol

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(ZEA), α-zearalenol (α-ZOL), β -zearalenol (β-ZOL) and de-epoxydeoxynivalenol (DOM-1) among adults in Sweden.

Keywords; concurrent exposure, diet, mycotoxins, urinary biomarkers, human biomonitoring

1. Introduction

Mycotoxins are naturally occurring secondary metabolites of fungi commonly found to contaminate large volumes of staple food (Magan and Olsen, 2004). Although there are geographical and climatic differences as well as substantial year to year fluctuations in the occurrence of mycotoxins in foodstuffs, exposure to these natural contaminants may be unavoidable (Bennett and Klich, 2003; Magan and Olsen, 2004). Mycotoxins are important from a health perspective as they display a wide range of deleterious effects, including hepatotoxicity, nephrotoxicity, teratotoxicity, heamatotoxicity, immunotoxicity and hormonal or reproductive effects but with different potencies depending on species and sex (Cortinovis et al., 2013; el Khoury and Atoui, 2010; Marin et al., 2013; Voss et al., 2007; Zinedine et al., 2007). Some mycotoxins, such as the main aflatoxins, aflatoxin B_1, B_2, G_1 and G_2 (AFB_1, AFB_2, AFG_1 and AFG_2) have been classified by the International Agency for Research on cancer (IARC) as carcinogenic to humans whereas OTA and FB_1 have been classified as possibly carcinogenic to humans (International Agency for Research on Cancer, 1993, 2002). Some mycotoxins, such as deoxynivalenol (DON), also cause acute effects and have been suggested as an etiological factor in several cases of human gastroenteritis (Bhat et al., 1989; Li et al., 1999; Yoshizawa et al., 1983).

However, humans are often exposed to more than one mycotoxin at the same time. This simultaneous exposure is due to several factors including the ability of some fungi to produce a number of mycotoxins simultaneously, as well as the fact that food commodities may be contaminated by several fungi. Moreover a varied diet consists of several food items and may

therefore be contaminated with a range of toxins. It is therefore relevant from a public health perspective to study simultaneous mycotoxin exposure. In recent years there has been a swift development in analytical methods resulting in the possibility of studying concurrent exposure through method assays capable of determination of concomitant mycotoxins in foods, as well as mycotoxin biomarkers in blood or urine (Capriotti et al., 2012; Solfrizzo et al., 2011; 2014; Warth et al., 2012; 2013). Biomarkers is a valuable tool in measuring exposure at the individual level while avoiding the problems associated with dietary registration and the heterogeneous contamination of mycotoxins in food which otherwise may hamper exposure assessments (Bryden, 2007; Solfrizzo et al., 2011; Timbrell, 1998; Turner et al., 2012; Turner et al., 2011).

Recently, a study concerning the levels of urinary DON showed that exposure to DON was very common (>90%) in the Swedish adult population (Wallin et al., 2013). To further explore the levels and possible patterns of mycotoxin exposure among the Swedish population, a multi-biomarker assay including six different mycotoxins or metabolites (aflatoxin M_1 (AFM_1), DON, DOM-1, FB_1, FB_2, NIV, OTA, ZEA, α-ZOL and β-ZOL) was used. Consumption data from the latest Swedish national survey investigating dietary habits among adults, Riksmaten 2010-11 (National Food Agency, 2012), was used in this study to analyse dietary patterns associated with the number of mycotoxins found in the urinary samples.

2. Material and methods

2.1 Study design and population

The study population took part in Riksmaten 2010-11, the latest Swedish national survey (National Food Agency, 2012) investigating dietary habits among adults. The Riksmaten study was conducted over one year, between May 2010 and July 2011, to capture seasonal

variations. Participants completed a self-assisted web based diet record over four consecutive days and a questionnaire covering background information and information about foods less frequently eaten. The participants also donated spot samples of urine which were collected on average 19 ± 34 days after the first day of dietary recording. Urine samples were collected at the Occupational and Environmental Medicine Centers in seven regions in Sweden. An equal number of individuals were randomly selected in each region independent of the population size. In total, 300 individuals provided spot urine samples (participation rate 30%). All participants gave oral informed consent before entering the study. The study was approved by the regional ethical committee in Uppsala (National Food Agency, 2012). The study population, dietary registration and sample collection is described in detail by Bjermo and collaborators and Wallin and collaborators (Bjermo et al., 2013; Wallin et al., 2013).

2.2 Urine analysis

Samples were sent frozen from the regional centres and distributed via the National Food Agency (Uppsala, Sweden) for analysis. The samples were sent by courier to the Institute of Sciences of Food Production (ISPA) in Bari (Italy) in May 2013 for mycotoxin (DON, NIV, FB_1, FB_2, ZEA, and OTA) and metabolite (AFM_1, DOM-1, α-ZOL and β-ZOL) analysis. Due to insufficient amounts of urine or miscoding only 252 samples were included for further analyses of the results.

2.3 Determination of urinary mycotoxin biomarkers

Urinary biomarkers (DON, DOM-1, AFM_1, FB_1, ZEA, α-ZOL, β-ZOL and OTA) were determined as previously described (Solfrizzo et al., 2011; 2014) with some modifications. Briefly, a 5 mL urine sample, instead of 6 mL, was incubated at 37°C for 18 h with 250 µL of β-glucuronidase/sulfatase type H-2 from *Helix* pomatia (Sigma Aldrich, Milan, Italy), diluted with 5 mL of water, instead of 6 mL, and purified on a Myco6in1^{+TM} IAC (Vicam, Watertown,

MA, USA) and OASIS® HLB (Waters, Milford, MA, USA) SPE column connected in tandem. The OASIS® HLB column was previously conditioned by passing 2 mL MeOH and 2 mL ultrapure water. After sample application and elution, the two columns were separated, the Myco6in1^{+TM} was washed with water (4 mL, discarded portion) and eluted with methanol (3 mL) and water (2 mL) and collected in a vial. The OASIS® column was washed with methanol/water (20:80, 1 mL) and was discarded. DON that had passed through the Myco6in1^{+TM} and retained on the OASIS® was eluted with methanol/water (40:60, 1 mL) that was collected in the vial containing the eluates from the Myco6in1^{+TM} column. The combined eluates were dried under air stream at 55°C and reconstituted in methanol/water (20:80, 200 µL) containing 0.5% acetic acid. Purified extract was filtered through a Minisart® RC 4 syringe filter (Sartorius, Muggiò, MB, Italy) and a volume of 10 µL (equivalent to 0.25 mL urine sample) was analyzed by UPLC-MS/MS.

The analyses were performed on a triple quadrupole API 5000 mass spectrometer (Applied Biosystems, Foster City, CA, USA), equipped with a ESI interface and an Acquity UPLC system comprising a binary pump and a microautosampler from Waters (Milford, MA, USA). The analytical column was an Acquity UPLC BEH phenyl column (2.1 x 150 mm, 1.7 µm particles; Waters) set at 40°C. The flow rate of the mobile phase was 250 µL/min. For multi-biomarker separation a linear gradient of MeOH (containing 0.5% acetic acid) in water (containing 0.5% acetic acid) was used as mobile phase as follows: 20 to 80% MeOH in 5 min, 80% MeOH for 5 min, 80% to 20% MeOH in 0.5 min and left to equilibrate for 4.5 min before the next run. For UPLC-MS/MS analyses, the ESI interface was used in positive ion mode for AFM_1, FB_1 and OTA and in negative ion mode for DON, DOM-1, ZEA, α-ZOL and β-ZOL.

Due to the cross reactivity of Myco6in1^{+TM} for NIV and FB_2, these mycotoxins were included in the panel of analytes determined by UPLC-MS/MS and were monitored in negative and

positive mode, respectively. The mass spectrometer operated in MRM (multiple reaction monitoring) mode. MS/MS conditions of all analytes are reported elsewhere (Lattanzio et al., 2014; Lattanzio et al., 2007). The biomarkers were quantified using matrix-matched standards and results were corrected for method recovery. To prepare matrix-matched calibration solutions, urines from 21 individuals were mixed and 5 mL aliquots were purified according to the protocol reported above. After purification, the combined eluates were spiked with appropriate amounts of each standard solution. The spiked extracts were dried down and reconstituted in 200 µL of methanol/water (20:80) with 0.5% acetic acid and filtered through a Minisart® RC 4 syringe filter. The ranges of equivalent biomarker concentrations in urine for the five matrix-matched calibration solutions were: DON 0.2 – 120.4 ng/mL, DOM-1 0.89 – 29.82 ng/mL, AFM_1 0.01 – 7.00 ng/mL, FB_1 0.01 – 12.19 ng/mL, ZEA 0.01 – 12.00 ng/mL, α-ZOL and β-ZOL 0.04 – 24.72 ng/mL, OTA 0.006 - 5.010 ng/mL. NIV and FB_2 were quantified with a single point of matrix-matched calibration solution specifically prepared for these two mycotoxins. Urinary creatinine was determined with Test™ Creatinine Enzymatic (Instrumentation Laboratory, Milan, Italy).

The analytical method used in the present study is a multi-biomarker method designed to include six mycotoxins (DON, ZEA, FB_1, FB_2, OTA & NIV) and four metabolites (AFM_1, DOM-1, α-ZOL & β-ZOL). All samples with detectable levels and <LOQ were considered as positives. When calculating the mean in all samples, values reported as <LOQ were assumed to be at LOQ. In the assessment of concurrent mycotoxin exposure, metabolites were considered as an indicator of exposure to the mother mycotoxin, i.e. samples where α-ZOL or β-ZOL were present but not ZEA were regarded as samples positive for the mother mycotoxin.

2.4 Statistical Analyses

The mycotoxins and metabolites as well as most food groups were skewed variables and non-parametric tests were used. The food groups analysed in this study were chosen based on previously reported associations with mycotoxins exposure (Magan and Olsen, 2004; Scientific Cooperation, 2003) and were the following; Legumes and pulses, Offal and blood courses, Poultry, Pork (domestic and wild bore), Nuts and seeds, Dried fruit, Coffee, Wine, Chocolates, Rice and grains, Corn and Cereal grain (flour). The cereal grain (flour) intake variable was created from the consumption data as previously reported (Wallin et al., 2013). In order to capture a high intake of foods potentially contaminated with mycotoxins a mycotoxin-diet-score was created, using data from the diet record which was available from 237 of 252 participants. For each of the food group described above, a reported intake below and above the median intake in all participants was coded as 1 and 2, respectively. Zero consumers were treated as 0. The mycotoxin-diet-score was created as the sum of scores for all food groups. The mycotoxin-diet-score ranged between 0 and 24 and was treated as an ordinal variable in the analyses. Since spot urine samples were used in this study, creatinine adjusted levels of mycotoxins and metabolites were applied in the statistical analysis.

Additionally, all participants were grouped according to the number of detected mycotoxins in the urine samples. The grouping variable had three levels; 0-1 mycotoxin, 2 mycotoxins and 3-5 mycotoxins and the groups are referred to as the low, medium or high multi-toxin group. Overall differences in body mass index (BMI), age, dietary characteristics and the level of creatinine adjusted mycotoxin and metabolite (ng/mg) between the multi-mycotoxin groups were investigated with ANOVA or Kruskal-Wallis rank test and then followed by t-test or Wilcoxon rank-sum test to find which groups were different from each other. Differences in mycotoxin levels between men and women, regions and seasonal variation

were checked with Kruskal-Wallis rank test. All analyses were carried out in STATA version 12.1 (STATA Corp. College Station, Tx, USA).

3. Results

The present study included analyses of six mycotoxins and three mycotoxin metabolites found in detectable levels (Table 1). Unfortunately, due to co-elution of an unknown interfering compound no results could be obtained for AFM1, resulting in a total of nine analytes to be included in the study. The present study revealed the presence of mycotoxins or mycotoxin metabolites in 249 out of 252 urine samples (99 %). The most frequently detected mycotoxin was DON followed by OTA and ZEA (Table 1). DOM-1 and NIV were only detected in samples where DON was also present.

Table 1.
Occurrence (n (%)) and levels of mycotoxins and metabolites;unadjusted (ng/ml) or adjusted for creatinine (ng/mg) in urine samples from Swedish adults.

Analyte	Number of positive samples[1]	Mean± SD in all (n=252) samples[2]	Mean ± SD in positive samples	Mean ±SD in all (n=252) creatinine adjusted samples	Mean ± SD in positive creatinine adjusted samples
	n (%)	(ng/ml)	(ng/ml)	(ng/mg)	(ng/mg)
DON	158 (63%)	3.37 ±10.1	5.38± 12.3	4.40 ± 19.6	7.02±24.4
DOM-1	20 (8%)	0.18 ±0.97	2.32± 2.68	0.12±0.55	1.56±1.26
ZEA	92 (37%)	0.03 ±0.06	0.09± 0.07	0.05±0.11	0.14±0.13
α-ZOL	53 (21%)	0.03 ±0.13	0.13± 0.26	0.04±0.14	0.19±0.26
β-ZOL	45 (18%)	0.02 ± 0.09	0.10± 0.19	0.02±0.09	0.13±0.18
FB_1	15 (6%)	0.004 ± 0.022	0.07± 0.06	0.01±0.07	0.13±0.26
FB_2	57 (23%)	0.01 ± 0.06	0.06± 0.12	0.04±0.25	0.16±0.51
OTA	128 (51%)	0.46 ± 0.57	0.90± 0.50	0.73±1.43	1.43±1.74
NIV	42 (17%)	0.02 ± 0.74	0.13± 0.14	0.02±0.05	0.12±0.07

[1] All samples with detectable levels, also <LOQ, were considered as positives.
[2] Mean in all samples, values reported as <LOQ are assumed to be at LOQ.
n number of positive samples.

Overall, creatinine adjusted levels of mycotoxins in the positive urine samples were greater in women than in men. Levels of ZEA, α-ZOL, β-ZOL, FB_2 and OTA were significantly higher

in women than in men (data not shown). The difference between men and women was particularly apparent for FB_2, ZEA and β –ZOL, where the mean level was nine, two and three times greater in women compared to men (p <0.001 for all).

Concurrent exposure to more than one mycotoxin was found in 173 samples (69%). The maximum number of mycotoxins found in a single sample was five (DON, ZEA, FB_1, FB_2, OTA). Most samples contained between one and three different mycotoxins (Fig. 1). There were no differences among men and women regarding the number of mycotoxins detected (p =0.98).

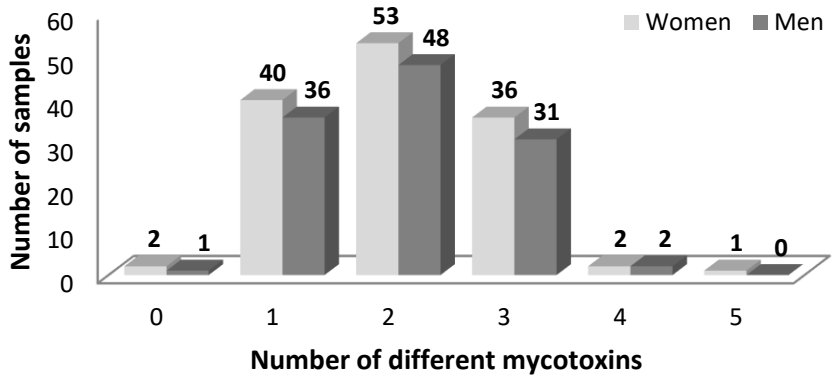

Fig. 1. Distribution of the number of different mycotoxins in the urinary samples of Swedish adults

Detectable levels of two concurrent mycotoxins were found in 101 samples (40%). The most common combinations were DON+OTA and ZEA+FB_2 which were found in 54 (21%) and 39 (15%) samples, respectively. Among the 67 samples (27%) with three different detectable mycotoxins, DON and OTA together with an additional toxin were found in 51 samples (76% of samples containing 3 mycotoxins). The most common combination was DON+OTA+NIV which was found in 38 samples (57% of samples containing three mycotoxins), as shown in Fig. 2.

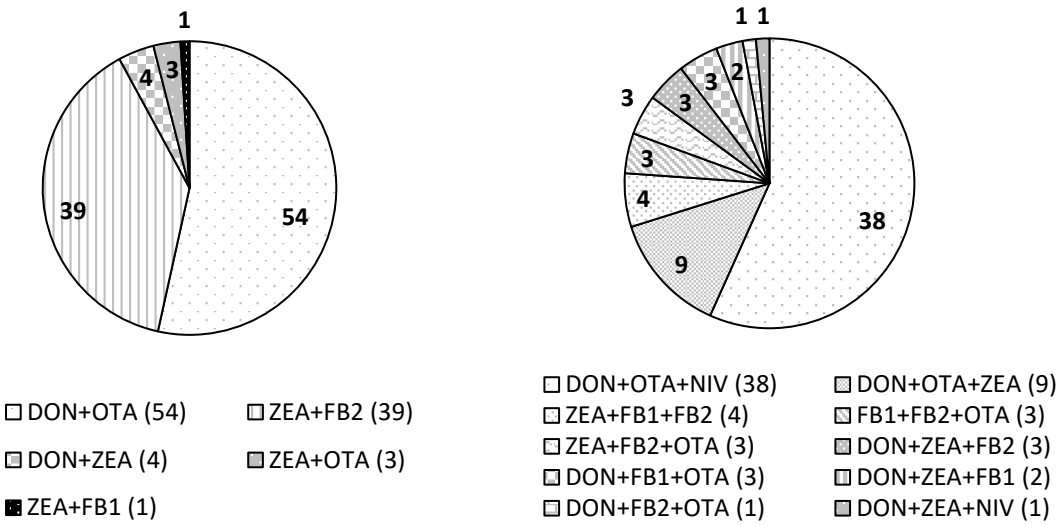

Fig. 2 Mycotoxin combinations and number of samples with 2 or 3 concurrent toxins.

When the levels of creatinine adjusted mycotoxins and metabolites (ng/mg) in positive samples were compared between the three multi-toxin groups, only the levels of DON and α-ZOL were significantly different between groups. DON levels were higher with a higher number of concomitant mycotoxins and metabolites (p <0.001) while α-ZOL levels were lower with a higher concomitant exposure (p =0.01). NIV was only present in the group with three or more concurrent mycotoxins. The levels of creatinine adjusted mycotoxins and metabolites were not different between geographical regions or seasons (data not shown). The number of individuals with zero mycotoxins detected in the present study was only three, thus excluding them from the statistical analyses had no impact on the results.

Table 2.
Background characteristics and nutrient intake in the study population for each multi-toxin group. The groups are based on the number of detected mycotoxins (0-5).

Multi-mycotoxin group (No of concurrent toxins)	Low multi-toxin group (0-1)			Medium multi-toxin group (2)			High multi-toxin group (3-5)		
Background characteristics	**n**	***Mean***	***SD***	***n***	***Mean***	***SD***	***n***	***Mean***	***SD***
Age (year)	79	47	17	101	52	17	72	48	16
BMI (kg/m^2)[a, b]	74	24.9	4.5	95	25.5	3.6	64	26.5	4.3

Number of days between urine sample and food record	77	17.2	37.7	95	21.2	40.7	65	19.5	16.7
Nutrient intake		***Median***	***IQR***		***Median***	***IQR***		***Median***	***IQR***
Energy (MJ)	77	8.6	7.0;1.3	95	8.6	6.8; 10.3	65	8.3	6.8; 10
Carbohydrate (E%)	77	43.1	37.5; 48.9	95	43.1	39.2; 47.2	65	45.3	41.0; 49.0
Total fat (E%)	77	34.5	31.3; 40.0	95	34.4	30.4; 39.0	65	32.8	30.0; 37.5
Saturated fat (E%)[a]	77	14.0	11.9; 15.5	95	12.8	10.8; 14.5	65	12.6	11.3; 13.8
Monounsaturated fat (E%)	77	12.7	11.4; 14.7	95	12.8	10.9; 14.9	65	12.2	11.0; 13.3
Polyunsaturated fat (E%)	77	5.3	4.3; 6.8	95	5.3	4.4; 7.1	65	5.5	4.4; 6.5
Protein (E%)	77	15.7	14.4; 17.7	95	16.3	15.2; 17.9	65	16.3	14.6; 18.7
Added sugar (g/day)	77	48.2	29.1; 67.0	95	42.9	32.4; 63.8	65	44.5	28.1; 66.5
Whole grain (g/day)	77	34.8	24.3; 52.5	95	49.3	22.2; 66.1	65	36.8	22.3; 60.9

n number of individuals

[a] Overall p <0.05.

[b] Body mass index (BMI) differed between the low and the high exposure group, p =0.01

SD standard deviation

IQR interquartile range

Table 3.

Intake of food groups and calculated mycotoxin-diet-score in the study population for each multi-toxin group. The groups are based on the number of detected mycotoxins (0-5).

Multi-toxin group (No of concurrent toxins)			Low multi-toxin group (0- 1) n=77		Medium multi-toxin group (2) n=95		High multi-toxin group (3-5) n=65	
Dietary characteristics Food groups		**N**	***Median***	***IQR***	***Median***	***IQR***	***Median***	***IQR***
Legumes and pulses (g/day)		118	0	0; 9.8	0	0; 24.8	1.6	0; 17.4
Offal and blood courses (g/day)		9	0	0; 0	0	0; 0	0	0; 0
Poultry (g/day)		130	10.5	0; 31.0	14.8	0; 39.9	4.5	0; 30.0
Pork[a](g/day)		218	16.7	5.0; 25.6	21.7	12.3; 41.5	21.0	7.5; 54.1
Nuts and seeds (g/day)		71	0	0; 0.5	0	0; 9.5	0	0; 0.8
Dried fruit[a](g/day)		101	0	0; 4	0.2	0; 6	0	0; 0.95
Coffee[a](g/day)		191	331	100; 513	413	200; 556	240	0; 469
Wine (g/day)		106	0	0; 100	0	0; 87.5	0	0; 87.5
Chocolate (g/day)		10	0	0; 12.5	0	0; 11.3	0	0; 10
Rice and grains (g/day)		104	0	0; 44	8.8	0; 43.8	0	0; 26.3
Corn (g/day)		31	0	0; 0	0	0; 0	0	0; 0
Cereal grains† (g/day)		233	111	85; 168	141	93; 175	135	92; 167
Mycotoxin-diet-score[b]		**237**	**10**	**7; 13**	**12**	**10;14**	**11**	**8;13**

[a] p <0.05, [b] p <0.01

n number of individuals

N number of consumers

† as defined in Wallin et al 2013, this variable excluded food items based on rice.
IQR interquartile range.

Differences in dietary and other characteristics between the multi-toxin groups are presented in tables 2 and 3. There were no differences between women and men in BMI (p=0.23). However, the women were younger than the men (p =0.002) and the mean age ± sd was 46 ± 1.4 and 53 ± 1.5 years in women and men, respectively (data not shown). Interestingly, BMI was significantly higher with a higher number of concomitant mycotoxins (p =0.01). There were no differences in age between the individuals in the multi-toxin groups (Table 2). There were only small differences in dietary characteristics between the three multi-toxin groups (Table 2 and 3). However, there was an overall difference between the groups regarding intake of saturated fat (p =0.03), pork (p =0.05), coffee (p =0.04) and dried fruit (mainly raisins, p =0.01). The mycotoxin-diet-score was also different (p <0.01), with the highest score in the medium multi-toxin group which was significantly different from both the low and the high multi-toxin groups (p <0.05 and p <0.01 respectively). When differences between each group were investigated, intake of saturated fat was significantly higher while intake of pork was significantly lower in the low multi-toxin group compared to the two other groups for (p <0.05). Consumption of coffee and dried fruits was lowest in the high multi-mycotoxin group (p <0.05 and p <0.01, respectively) when compared with the medium multi-mycotoxin group. In the medium multi-toxin group the intake of whole grains, poultry, and cereal grains (flour) was numerically the highest although there was no overall significance for these groups.

4. Discussion

The present study investigates for the first time individual and concurrent mycotoxin exposure to FB_1, FB_2, NIV, OTA and ZEA, α-ZOL, β-ZOL and DOM-1 in Swedish adults.

The results clearly show that exposure to mycotoxins is common among adults in Sweden (Table 1).

DON was the most common mycotoxin detected in the urine (Table 1), suggesting that foods contaminated with DON are frequently consumed. DOM-1 was only found in samples also containing DON, suggesting that humans have the ability to detoxify DON by this route. Surprisingly, NIV was also only found in samples containing DON, but the reasons for this remain unclear.

Half of the study participants had detectable levels of OTA (Table 1), which is in agreement with results from other European countries (Duarte et al., 2011; Malir et al., 2012). In an earlier study where OTA was analyzed in blood plasma from Swedish plasma donors, large regional differences were identified. Much higher exposure rates (30-100%) were found on an island in Baltic Sea compared to the mainland (3-6%) (Breitholtz et al., 1991). However, in the present study neither regional nor seasonal differences could be identified for OTA.

ZEA was detected in over one third of the samples. Although ZEA is of relatively low acute toxicity (Zinedine et al., 2007), it is of interest to monitor exposure since ZEA or derivatives thereof have oestrogenic properties (Minervini et al., 2005) and has been implicated as an underlying cause of early onset pubertal changes in children (Saenz de Rodriguez et al., 1985; Szuets et al., 1997). ZEA has also been suggested to be a part of the aetiology of human breast cancer (Yu et al., 2005). Interestingly levels of ZEA, α-ZOL and β-ZOL in this study were significantly higher in women compared to men ($p < 0.001$ for all).

The high number of samples positive for FB_2 as compared to FB_1 (Table 1) contrasts with previous results (Abia et al., 2013; Ezekiel et al., 2014; Riley et al., 2012). The reasons for

this discrepancy are not clear and interpretation of results is further hampered by the low proportion of FB_1 excreted in urine (Riley et al., 2012; van der Westhuizen et al., 2011). However, it should be mentioned that FB_2 but not FB_1 has previously been found in samples of must, raisins and coffee due to contamination of *Aspergillus niger* which is a fungus that produces FB_2 but not FB_1 (Frisvad et al., 2007; Logrieco et al., 2009; Noonim et al., 2009; Perrone et al., 2013).

Concurrent exposure to more than one mycotoxin was common in the study population (Fig. 1). Frequent co-exposure has been reported by other studies from the Transkei area in South Africa (Shephard et al., 2013) and South Italy (Solfrizzo et al., 2014). However, exposure to more than two mycotoxins was less frequent in Cameroon (18 %) and Nigeria (13%), (Abia et al., 2013; Ezekiel et al., 2014), which possibly relates to a lower sensitivity of the analytical method used, or by differences in dietary patterns.

The by far most common toxin combination found in the urine was DON + OTA. However, it is unclear what effects, if any, the particular mixture of DON and OTA may cause in humans and literature on the subject is scarce. In a study by Kubena and collaborators, which focused on the combination of DON and OTA in broiler chickens, interactions for many parameters were found to be less than additive, mainly reflecting the effect of one of the toxins without additional effect of the other toxin, or antagonistic (Grenier and Oswald, 2011; Kubena et al., 1988). Other common combinations found were ZEA+FB_2 in samples with two concurrent mycotoxins and NIV+DON in the samples with three concurrent mycotoxins (Fig. 2). Effects on toxicity from mixtures of *Fusarium* mycotoxins in various test systems are more frequent, but results may vary depending on tests system, doses, the ratio of toxins in the mixture, studied end-point etc (Alassane-Kpembi et al., 2013; Kouadio et al., 2007; Luongo et al., 2008; Tajima et al., 2002; Wan et al., 2013a; Wan et al., 2013b). In conclusion,

when several mycotoxins occur simultaneously in a mixture they may interact with each other in such a way that the resulting toxic effects may be difficult to predict. Moreover, concurrent exposure is of relevance even at low doses since negative effects may be caused by a combination of mycotoxins at amounts below the concentrations reported for the individual toxins to cause effects (Domijan et al., 2007).

This study also attempted to identify dietary characteristics associated with the number of mycotoxins present in the urine samples of the participants. However, only slight dietary differences except for the intake of saturated fat, pork, dried fruits and coffee intake were observed (Table 2 and 3). The lack of pronounced differences may be related to an uneven distribution of mycotoxins in foods as well as to the inherent limitation to study dietary exposure; including the difficulty to estimate portion sizes as well as reporting errors (Livingstone and Black, 2003; Macdiarmid and Blundell, 1998). Furthermore, the four day registration period in the present study may have been too short to capture usual intake of rarely consumed food groups, except for pork and coffee. Interestingly though, the highest mycotoxin-diet-score was found in the medium mycotoxin group, i.e. with 2 mycotoxins detected in the urine (Table 3). This may be due to the reasons discussed above or that some dietary sources contributing to the mycotoxin exposure were not included. Furthermore, there was no difference in the lag-time between urine sample and diet record between the multi-toxin groups (Table 2), which indicates that sampling time did not affect the results. That saturated fat intake was the highest in the low mycotoxin group is expected since a lower intake of cereal foods will consequently render a diet higher in fat and/or protein.

5. Conclusions

Exposure to mycotoxins is common in Swedish adults. The most frequently detected mycotoxin was DON, followed by OTA and ZEA. The study also revealed that concurrent

exposure is prevalent in the Swedish adult population. The most common toxin combination found in the urine of the study participants was DON together with OTA. However, to study the associations between specific food patterns and concurrent mycotoxin exposure was challenging. Further investigations of combined exposure to mycotoxins and associations with diets are warranted.

Conflict of interest

The authors declare that there are no conflicts of interest.

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