Environmental Research 242 (2024) 117618

Contents lists available at ScienceDirect

Environmental Research

journal homepage: www.elsevier.com/locate/envres

Associated factors with mycotoxin exposure in Spanish population

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ARTICLE INFO

Handling Editor: Jose L Domingo

Keywords:
Biomonitoring
Urine
Metabolites
Diet
Environment
HPLC-Q-TOF-MS

ABSTRACT

Human exposure to mycotoxins is a global concern since filamentous fungi can contaminate food and feed from crops to ready-to-eat meals. Human urine biomonitoring is a widely used technique to evaluate mycotoxins exposure, as an alternative to food correlation studies. The aim of this study is to describe human exposure to mycotoxins and to investigate the associated sociodemographic, lifestyle and dietary variables. Participants were 540 women from the Valencia (Spain) cohort of the Spanish Childhood and Environment Project (INMA). A validated multi-mycotoxin method using HPLC-Q-TOF-MS was applied to determine the concentration of ten selected mycotoxins: Enniatin A, Enniatin B, Enniatin A1, Enniatin B1, Beauvericine, Aflatoxin B1, Aflatoxin B2, Aflatoxin G1, Aflatoxin G2 and Ochratoxin A. A simultaneous untargeted screening of mycotoxins and their metabolites has been performed. Mycotoxins associations were assessed by bivariate and multivariate regression models using participants' sociodemographic, lifestyle and dietary data collected through questionnaires. Mycotoxins were detected in 81% of urine samples. The method quantified mycotoxins concentrations in up to 151 samples. Most quantified mycotoxins were: Enniatin B [% of detection (concentration range)] = 26% (1.0–39.7 ng/mg) and Enniatin B1 = 7% (0.5–14.4 ng/mg). Besides the ten-targeted mycotoxins, other mycotoxins and metabolites were studied, and higher incidence was observed for Deepoxy-deoxynivalenol (45%), Ochratoxin B (18%) and Ochratoxin *α* (17%). Higher mycotoxins concentrations were associated with rural areas as well as with participants belonged to lower social class, beer, light sodas and fruit juice consumers. On the contrary, higher processed meat intake was related to lower mycotoxins' levels. Studies are required to better evaluate the exposure to mycotoxins from food and their environmental relationships.

1. Introduction

Globalization and intensification of food production along with climate changes, are related to the presence of emerging contaminants in food products and environment, that represent a threat to human and animal health (Dupouy and Popping, 2022). Among this, mycotoxins are fungi toxic secondary metabolites widespread in crops and food products, being impossible nowadays to guarantee its absence in the market (Manyes and Font, 2023). In order to protect consumers' health and assure food safety, many countries have established maximum admissible levels for mycotoxins as ochratoxin A (OTA) and aflatoxins (AFs), among others, in several food products (European Commission, 2023). OTA's and AFs toxicological effects are well known such as inflammation, impaired neuronal differentiation, DNA damage, oxidative stress and cell death (Frangiamone et al., 2022). OTA and AFs are classified by the International Agency for Research on Cancer (IARC) as possibly carcinogenic to humans (group 2B) and carcinogenic to humans (Group 1), respectively (Claeys et al., 2020). Furthermore, *Fusarium* emerging mycotoxins, have been less considered for years, because of the low probability of acute toxicity, but their toxicological study has increased recently (Escrivá et al., 2015). *In vitro* and *in vivo* studies for enniatins (ENNs) and beauvericine (BEA) have shown genotoxic and cytotoxic

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https://doi.org/10.1016/j.envres.2023.117618
Received 28 September 2023; Received in revised form 6 November 2023; Accepted 7 November 2023
Available online 14 November 2023

effects. It has been observed that ENNs generated toxic effects in rats at mitochondrial level after being exposed to medium concentrations: single dose of ENA 256, ENA1 353, ENB 540, ENB1 296 μg/mL; and higher concentrations: single dose of ENA 513, ENA1 706, ENB 1021, ENB1 593 μg/mL. Toxic responses observed were changes in the epithelial barrier, suggesting a decrease of the permeability (Cimbalo et al., 2020, 2021).

Mycotoxins can contaminate food in different stages, since fungi can proliferate during harvest, storage, and in ready-to-eat food. It has been shown that temperature and water activity have an important impact in fungi growth (Romero Donato et al., 2022). Most mycotoxins suffer partial degradation but are not eliminated after food processing, being a food safety and economic concern for food industries (Urugo et al., 2023). Legislated mycotoxins are not expected to be found in high concentrations in food products, because of the strict regulations and hazard analysis critical control point strategy carried out in the industry (Gil et al., 2016). Otherwise, emerging mycotoxins represent a growing concern since its presence in food has been shown recently, as for *Alternaria* (alternariol monomethyl ether, alternariol, tentoxin, tenuazonic acid, and altenuene) and *Fusarium* (enniatin A (ENNA), enniatin A1 (ENNA1), enniatin B (ENNB), enniatin B1 (ENNB1), and BEA) mycotoxins, which were found in tomato products from China, with 58.8% of samples exhibiting at least one mycotoxin (Ji et al., 2023a). In Europe, commercial samples of bread loaves purchased from different supermarkets located in Valencia (Spain) were investigated for the presence of legislated and non-legislated mycotoxins. Results showed that samples were contaminated with AFs, zearalenone (ZEA) and ENs with a frequency of 20, 96, and 65% respectively (Saladino et al., 2017).

A previous study about the validation of an analytical method to detect and quantify mycotoxins in human urine using a subsample (n = 100) was carried out, finding relevant mycotoxin concentrations in 30% of the samples, being ENNs the most frequent ones (Dasí-Navarro et al., 2022). Human biomonitoring (HBM) studies are an interesting alternative to food consumption data correlation studies in order to assess mycotoxins exposure in humans through diet, which can also consider joint exposure from other factors as inhalation or dermal contact, only possible in professional ambiences (Martins et al., 2019). For this HBM studies urine is a relevant matrix to be used, since it is noninvasive and easy to collect (Foerster et al., 2021; Wang et al., 2022). Several mycotoxin exposure studies have applied HBM to determine urinary biomarkers, as in 439 pregnant women cohort in Bangladesh, where biomarkers for six major mycotoxins (AFs, citrinin (CIT), deoxynivalenol (DON), fumonisin B1 (FB1), OTA, and ZEA) were detected in urine samples, being the most frequently OTA (95%), CIT (61%), and DON (6%) and co-occurrence was observed in 63% of samples (Kyei et al., 2022). Also, in China a biomonitoring study was carried out to assess *Alternaria* mycotoxins' exposure in 269 urine samples, detecting AOH, AME, TeA and TEN in 38.3%, 48.7%, 63.9% and 23.4% of samples respectively (Fan et al., 2021).

Mycotoxins exposure can occur besides dietary intake, by dermal contact and inhalation but only under unappropriated working conditions. In swine production in Portugal has been found contamination in feed and litter samples, leading to occupational exposure of workers, due to specific work tasks involving inhalation of dust particles containing mycotoxins inhalation or dermal contact, being DON the most prominent mycotoxin in this samples (Viegas et al., 2019). Also, in swine feeding samples from Spain, 19 regulated and non-regulated mycotoxins (aflatoxin B1 (AFB1), aflatoxin B2 (AFB2), aflatoxin G1 (AFG1) and aflatoxin G2 (AFG2), OTA, FB1 and fumonisin B2 (FB2), CIT, ZEA, DON, fusarenon X (F-X), sterigmatocystin (STE), T-2 toxin (T-2), HT-2 toxin (HT-2), ENNA, ENNA1, ENNB and enniatin B2 (ENNB2), and BEA) were quantified and co-occurrence of up to five mycotoxins in a significant number of samples was detected. The most commonly found mycotoxins were the emerging ENNB (100% samples), BEA (93.4%), ENNB1 (53.5%), FB1 (50.0%) and ENNA1 (40.8%) (Arroyo-Manzanares et al., 2019).

The aim of this work is to describe exposure to regulated and emerging mycotoxins and the sociodemographic, lifestyle and dietary factors associated with the exposure in adult women urine samples from Valencia (Spain).

2. Materials and methods

2.1. Study population

Samples were obtained from women participating in the INMA (INfancia y Medio Ambiente-Environment and Childhood: https:www.proyectoinma.org), a multicenter birth cohort study that aims to study the exposure to environmental pollutants from air, water, and diet during pregnancy and childhood and the effect on child and adolescent growth and development in different geographical areas of Spain (Guxens et al., 2012). Women were recruited from the general population (n = 855) during their first antenatal visit at la Fe hospital (2003–2005) and followed up until birth (n = 787). Inclusion criteria included being a resident in the study area, being at least 16 years old, having a singleton pregnancy, not having followed any assisted reproduction program, desiring to deliver at the reference hospital, and having no communication difficulties. Their children were enrolled at birth and were monitored until they were 4 years of age. In the current study 540 urine samples were considered, collected from mothers when their children reached 4 years old (2007–2008).

Informed consent was obtained from all participants in each phase, and the study protocol was approved by the Public Health Research Centre in Valencia (CSISP) and La Fe hospital Ethics Committees.

2.2. Sociodemographic covariates

Sociodemographic, environmental and lifestyle data was collected by questionnaires administered by trained interviewers. The covariates used in this study were age (years), body mass index (BMI) (kg/m^2), country of birth (Spain, Latin America, other), education level (up to primary, secondary, university), type of zone of residence (urban, semi-urban, rural), working status (yes/no), and smoking (yes/no), and parity (0, 1, ≥2) which refers to the number of times the woman has given birth before the pregnancy that is the subject of the study, meaning 0 that the participant is primiparous.

We defined social class from the occupation according to a widely used Spanish adaptation of the International Standard Classification of Occupations coding system (ISCO88). Class I + II included managerial jobs, technical staff and commercial managers; Class III included skilled non-manual workers; and class IV + V included manual and unskilled workers (Domingo-Salvany et al., 2013).

2.3. Dietary covariates

Information on diet was obtained from a semi-quantitative food frequency questionnaire (FFQ). This FFQ was validated in the Valencia cohort with good reproducibility for nutrient and food intake (Vioque et al., 2013). Data was obtained (expressed as the mean of the daily servings) on the intake of dairy products, eggs, meat (including red and white meat categories), seafood (including lean fish, oily fish and others), fruit, vegetables, legumes, nuts, potatoes, cereals and pasta, bread, sweets, beverages with and without alcohol, coffee and other infusions (such as tea), animal and vegetal fats, processed food and dressings. Specific food from each food group was also used to assess later specific associations in case of the food group significance. Estimates on daily caloric intake were derived from the FFQ and food consumption was adjusted for calories accordingly.

2.4. Mycotoxins determination

2.4.1. Chemicals and reagents

Methanol and ACN, both LC/MS grade were obtained from Fisher Scientific (Loughborough, UK), and Formic acid (CH_2O_2, grade >98%) from Acros organics (Geel, Belgium). Deionized water was acquired at the laboratory through a Milli-QSP® Reagent Water System (Millipore, Bedford, MA, USA). Magnesium sulfate, anhydrous (>99.5%) ($MgSO_4$), was supplied by Thermo Fisher (Kandel, Germany). The C18-E (50 μm, 65 Å) HPLC column was acquired from Phenomenex (Torrance, CA, USA). Nylon syringe filters 13 mm 0.22 μm from Membrane Solutions (Valencia, Spain). Creatinine (98%) was purchased from Acros organics (Loughborough, UK), Picric acid ($C_6H_2OH(NO_2)_3$) (98%) from Panreac (Barcelona, Spain), and NaOH from VWR (Radnor, PA, USA).

2.4.2. Standards and solutions

The analytical standards were purchased from Sigma Aldrich (St. Louis, MO, USA) for ENNA, ENNA1, ENNB, ENNB1, BEA, AFB1, AFB2, AFG1, AFG2, and OTA, and with these standards it was prepared a combined standard working solution in methanol at 100 μg/mL. From the initial working mix, a series of dilutions were prepared, and all working solutions were stored at −20 °C protected from light.

2.4.3. Sampling

For this study, 540 urine samples were stored at −20 °C until analysis, and creatinine concentration was determined to normalize results, 16 samples with very low (<0.1 mg/mL) creatinine values were discarded, resulting in 524 samples selected to continue with the analysis. For the previous preparation of the sample, urines were centrifuged at 10,640×*g* for 10 min at 4 °C, then supernatant was gathered to perform the creatinine determination and the extraction method.

2.4.4. Simple QuEChERS extraction method

For sample extraction, it was employed an in-house previously validated method (Dasí-Navarro et al., 2022) which is based on Quick Easy Cheap Effective Rugged and Safe (QuEChERS) procedure. Linearity, precision, sensibility (limits of detection (LODs) and limits of quantification (LOQs)), recoveries, and signal suppression-enhancement (SSE, %) were assessed for method validation according to the Commission Decis (European Commission, 2002). Firstly, 1 mL of previously centrifuged urine was added to a tube with 0.3 g of $MgSO_4$ and 0.03 g of C18 and vortexed, then 1 mL of ACN was added and then vortexed for 1 min. Afterward, samples were centrifuged at 3200×*g* for 5 min at 4 °C. The upper layer containing the organic phase was separated and filtered with a nylon syringe filter of 13 mm 0.22 μm to an HPLC vial.

2.4.5. Creatinine determination

Creatinine determination was performed following the same methodology as in Dasí-Navarro et al. (2022), which principle is the production of a yellow-orange complex of creatinine and picric acid, whose absorbance is measured at 500 nm wavelength. For this measurement, a VWR UV-1600PC spectrophotometer was employed. After creatinine determination of the 540 samples, <0.1 ng creatinine/mL was considered to discard too diluted samples that could affect the values of mycotoxins concentrations. With this criteria, 16 samples were discarded, therefore for current study 524 samples were considered.

For results normalization, a creatinine-adjusted concentration of each mycotoxin (ng/mg) was calculated to obtain a more accurate index and facilitate comparison with the literature based on mycotoxin concentration of positive samples (ng/mL) and creatinine concentration (mg/mL). Creatinine concentration range in the selected urine samples was from 0.1 to 1.5 mg/mL (mean concentration 0.6 ± 0.2 mg/mL).

2.4.6. LC-Q-TOF-MS conditions

To perform the chromatographic analysis, it was employed an Agilent 1200 LC system (Agilent Technologies, Santa Clara CA, USA) coupled to a 6540 Ultra High Definition Accurate-Mass Q-TOF-MS and set up with the Dual Agilent Jet Stream Electrospray Ionization (Dual AJS ESI) source interface. The LC system was equipped with an autosampler, a binary pump and a degasser. For this work, the column employed was a Phenomenex (Torrance CA, USA) Gemini® NX-C18 (3 μm, 30 × 2 mm ID) with a guard column Security GuardTM Ultra C18. As mobile phases were selected water (A) and ACN (B), both with 0.1% of formic acid. Injection volume was 10 μL and flow was 0.3 mL/min. The gradient of mobile phases started at 0 in phase B and increased linearly to 3% in 3 min; after, phase B increased to 50% in 4 min; then, phase B increased to 75% in 5 min. After holding for 3 min, it returned to the initial parameters in 3 min. Resulting in a total run time of 18 min. The ionization mode selected for the MS detection was in positive with the following settings: sheath gas temperature of 300 °C, sheath gas flow 12 L/min, drying gas flow (N_2) 5 L/min; gas drying temperature 325 °C; nebulizer pressure 60 psi. Ion source parameters were: nozzle voltage 1200 V, capillary voltage 2500 V, fragmentor voltage 170 V, skimmer voltage 70 V and Oct1RFVpp 750 V. Collision energy voltage for fragmentation was 10 V. Regarding spectral parameters, acquisition was performed in the mass range 100–900 m/z at a scan rate/time of five spectra/s. An internal mass correction was performed with reference masses 121.0509 and 922.0098 m/z.

Besides urine samples, to guarantee the correct operation of the instrument during sample analysis, matrix-matched and standard calibration curves were injected as routine.

2.4.7. Targeted mycotoxins quantification

Agilent Personal Compound Database Library (PCDL) Manager including Metlin mass spectral library data was employed to process peak results, creating a custom database which included the ten validated mycotoxins and other mycotoxins and metabolites for untargeted analysis. For mycotoxins and metabolites identification, Agilent Mass Hunter Workstation Software was employed by comparing molecular mass spectra of the samples with those in the PCDL Metlin database, and then manual integration of peak chromatograms was made for authentication of the compounds. To verify the presence of the validated mycotoxins (ENNA, ENNA1, ENNB, ENNB1, BEA, AFB1, AFB2, AFG1, AFG2, and OTA) in the positive samples, retention time (RT), mass, and m/z were compared with values obtained by standards injection and biochemical databases. Following this, those mycotoxins with results above LOQ, were accurately quantified through matrix-matched calibration curves for each targeted mycotoxin.

2.4.8. Screening for untargeted identification

Simultaneously to the validated mycotoxins identification procedure, also other mycotoxins and metabolites were identified by a screening study carried out for the aforementioned software. This screening was made by adding the parent mycotoxins and metabolites of interest to the custom PCDL database (diacetoxyscirpenol (DAS), HT-2, altenuene (ALT), neosolaniol (NEO), ZEA, DON, AOH, FB1, deepoxy-deoxynivalenol (DOM-1), 15-acetyl-deoxynivalenol (15ADON), 3-acetyl-deoxynivalenol (3ADON), deoxynivalenol-3-glucoside (DON-3-G), ochratoxin B (OTB), ochratoxin α (OTα), α zearalenol (α-ZOL), aflatoxin M1 (AFM1), aflatoxin M2 (AFM2), and aflatoxin P1 (AFP1). With Agilent Mass Hunter Workstation Software is possible to perform an untargeted scan of unknown analytes through a full spectra screening of the sample, and then identifying compounds with the pre-sett (Martins et al., 2020). Mycotoxins and metabolites results provided by the software with scores higher than 70%, were qualified as true identified compounds.

2.5. Statistical analysis

Basic descriptive statistics were calculated for the 524 participants for each mycotoxin scenario of exposure (mycotoxins concentration, presence/absence of any mycotoxin or metabolite detected and number

of detected mycotoxins or metabolites).

Associated factors analyses were carried out considering only the participants with complete information about their sociodemographic and dietary covariates. Bivariate and multivariate regression models were built, as in other studies related to the INMA project (López-González et al., 2023; Soler-Blasco et al., 2019, 2021), to examine the association between mycotoxins exposure and sociodemographic, environmental, and dietetic factors. Linear regression models with Gaussian distribution were used in case of the total ENNs concentrations outcome, with Poisson distribution for number of detected mycotoxins or metabolites outcome, and logistic regression models for the presence/absence of any mycotoxin or metabolites. These models were built in a two-step procedure. First, a bivariate analysis was run considering all the sociodemographic, lifestyle and dietary covariates. All covariates showing a p-value <0.20 in the likelihood ratio test (LRT) were included in the multivariate model and a backward elimination procedure was run. Covariates with p-value <0.10 were retained in the model. All models were adjusted for maternal age. In case of any food group significance, each specific food item from the group was assessed in the models in order to identify the specific foods associations.

All analyses were performed using R software (4.2.2 version). Associations at $p < 0.05$ were considered statistically significant, and associations at $p < 0.10$ as marginally significant.

3. Results

3.1. Analysis of mycotoxins in urine samples

Among the 524 samples, at least one mycotoxin was detected in 423 samples (81%). Results for determination and quantification of the 10 targeted mycotoxins are shown in Table 1. To obtain a more accurate index and facilitate comparisons, a mycotoxin creatinine-adjusted concentration (ng mycotoxin/mg creatinine) was performed, based on mycotoxin urine concentration (ng/mL) and creatinine urine concentration (mg/mL). At least one mycotoxin was detected, and then quantified, in 151 samples (29%). Most quantified mycotoxins were: ENNB [% of detection (concentration range)] = 26% (1.0–39.7 ng/mg) and ENNB1 = 7% (0.5–14.4 ng/mg). Only ENNs concentrations reached enough incidence to carry out regression analyses so a new variable was calculated with the sum of the different ENNs concentrations (ΣENNs, mean ± SD = 5.2 ± 6.4 ng/mg).

Besides targeted identification, to determine the occurrence of other mycotoxins present in the samples, a screening study was performed, employing the custom Metlin PCDL which besides the 10 targeted mycotoxins, included some other 18 mycotoxins and metabolites. According to this, results for identified mycotoxins (with >70% score) are presented in Table 2. Higher incidence was observed for DOM-1 (45%), OTB (18%) and OTα (17%).

Table 1
Incidence and creatinine corrected concentration (ng/mg) of targeted mycotoxins in studied samples (n = 524).

Mycotoxins	Nº of detected samples	% of detected samples	Concentration range (ng/mg)	Mean ± SD
ENNB	134	26	1.0–39.7	4.8 ± 4.8
ENNB1	35	7	0.5–14.4	2.1 ± 2.7
ENNA	8	2	0.3–2.1	0.7 ± 0.6
AFG1	7	1	1.1–9.1	3.0 ± 2.8
AFB1	4	1	0.6–1.2	1.0 ± 0.3
AFB2	4	1	0.6–9.9	3.9 ± 4.1
ENNA1	3	1	1.1–2.8	2.0 ± 0.9

Table 2
Data base screening of untargeted mycotoxins in studied samples (n = 524).

Detected mycotoxins	Nº of detected samples	% of detected samples
DOM-1	238	45
OTB	95	18
OTα	91	17
NEO	38	7
α-ZOL	35	7
DON	28	5
ZEA	21	4
15-ADON/3-ADON[a]	19	4
HT-2	14	3
DAS	9	2
ALT	8	2
AFP1	5	1
DON-3-glucoside	3	1
AOH	2	<1
AFM2	2	<1
FB1	2	<1
FB3	1	<1

[a] 15-ADON and 3-ADON have very similar structure and the same signal in the instrument, and since the purpose of this study was not to distinguish between them, both were considered as a sum.

3.2. Associated factors to mycotoxins exposure

3.2.1. Study population

For this work, within the 423 positive samples, only those with participants' information about their sociodemographic and dietary covariates complete, were employed for associated factors analysis, being 349 samples for the "Presence of any mycotoxin" exposure variable and 97 samples for the "Sum of ENNs" variable. Mother's characteristics are shown in Table 3, which includes descriptives of the associated factors evaluated in the samples. Body weight ranged from 50 to 93 kg (mean 64.5 ± 12 kg) and mean age was 35.6 ± 4 years. For both exposure variables most of the participants were from Spain (>90%). Regarding educational level and social class, it is observed that there is a higher percentage of participants with primary and secondary studies and lower social class (SC IV + V). About the residence area, more than 80% of the participants live in urban areas.

3.2.2. Bivariate analysis

Bivariate analysis showed that higher ENNs levels were related to living in urban or semiurban areas, non-alcoholic drinks, blue fish, and pasta consumption. Participants with secondary or university studies, lower social class, as well as sausages, fruits and potatoes intake, were highly associated with the determination of any mycotoxin in the samples. Secondary and university studies, lower social class, as well as vegetables, alcoholic drinks and blue fish consumption, were associate with higher number of mycotoxins and metabolites detected (Supplementary material).

3.2.3. Multivariate analysis

Fig. 1 shows the results for the linear regression models which indicates an inverse relationship between ENNs concentrations and living in semi-urban or urban zones (β [CI 95%] = −7.574 [(−12.793)-(-2.355)] and 7.491 [(−11.593)-(-3.388)], respectively), in comparison with rural areas, also a negative association with smokers was observed (−2.571[(−4.974)-(-0.167)] and a positive relation with the non-alcoholic beverages intake (3.034 [1.217–4.852]. The specific foods associated with ENNs exposure were light sodas (0.013 [0.001–0.025]) and fruit juice (0.01 [0.0–0.019]).

Fig. 2 shows the results of the logistic regression models for the associated factors to the mycotoxins and metabolites determination (presence/absence), observing positive associations of mycotoxins presence with lower social class (3.311 [0.365–2.030]), high school

Table 3
Characteristics of the population included in the associated factors analysis.

Exposure variable	Population covariates	Participant (%)	p value (Kruskal-Wallis)
Birth country			
Sum of ENNs	All	97 (100%)	0.844
	Spain	89 (92%)	
	Other	8 (8%)	
Presence of any mycotoxin	All	349 (100%)	0.437
	Spain	319 (91%)	
	Other	30 (9%)	
Education			
Sum of ENNs	All	97 (100%)	0.618
	Up to primary	41 (42%)	
	Secondary	33 (34%)	
	University	23 (24%)	
Presence of any mycotoxin	All	349 (100%)	0.003
	Up to primary	103 (30%)	
	Secondary	148 (42%)	
	University	98 (28%)	
Social class			
Sum of ENNs	All	97 (100%)	0.557
	SC I + II (highest)	17 (18%)	
	SC III	27 (28%)	
	SC IV + V	53 (55%)	
Presence of any mycotoxin	All	349 (100%)	0.129
	SC I + II (highest)	89 (26%)	
	SC III	98 (28%)	
	SC IV + V	162 (46%)	
Living area			
Sum of ENNs	All	97 (100%)	0.355
	Rural	8 (8%)	
	Semiurban	10 (10%)	
	Urban	79 (82%)	
Presence of any mycotoxin	All	349 (100%)	0.878
	Rural	15 (4%)	
	Semiurban	43 (12%)	
	Urban	291 (84%)	
Parity			
Sum of ENNs	All	97 (100%)	0.570
	0	51 (53%)	
	1	37 (38%)	
	≥2	9 (9%)	
Presence of any mycotoxin	All	349 (100%)	0.912
	0	192 (55%)	
	1	130 (37%)	
	≥2	27 (8%)	

education (2.796 [0.324–1.733]) and university education (1.893 [−0.201-1.477]). Statistically significative foods were fruits (1.449 [(−0.072)-0.813]) and potatoes (1.339 [(−0.057)-0.641]) with positive association, and sausages group (0.734 [(−0.630)-0.011] with a negative association.

Fig. 3 shows the results of the linear regression models employed to assess associated factors to the increasing number of detected mycotoxins and metabolites. Lower social class (1.479 [0.091–0.691]) and alcoholic beverages (1.040 [(−0.001)-0.080]) consumption were related to higher number of detections. In this case, beer intake showed the significant relationship (0.003 [0.001–0.005).

4. Discussion

4.1. Mycotoxin exposure in urine samples

In the present study, DON or its metabolites were detected in 51% of samples, finding higher determinations for DOM-1 than for DON. Obtained results for this study are in accordance with previous HBM studies employing human urine samples, where DON and its metabolites where the most frequently detected biomarkers in 24 h urine samples, with 41% of positive samples for DOM-1 and 63% for DON (Martins et al., 2019). In humans and animals DON is metabolized to DOM-1 and excreted in feces and urine (De Santis et al., 2019). DON has a short biologic half-life and is rapidly cleared from bloodstream, first morning samples show exposure from previous dinner but not overall daily exposure (Wang et al., 2022). Kyei et al. (2022) and Warensjö Lemming et al. (2020) also found low incidence of DON 6% and 4.8% respectively in first urine samples. Type of sampling could be the reason for finding more DON in its metabolized form, but in any case, the incidence of total DON is higher than other mycotoxins, agreeing with other authors (De Santis et al., 2019).

Regarding OTA, it has a long half-life and only a small fraction goes through glomerular filtration and urinary excretion, OTα is the major metabolite of OTA in humans and is more efficiently cleared from circulation and excreted with urine (Ali et al., 2017). In the present study, no OTA was detected but its metabolites OTα and OTB were the second most detected biomarkers, with a joint incidence of 32%. Collins et al. (2021) detected OTA in 71% of Rwanda population samples but employing Solid Phase Extraction (SPE) cartridges which reduces matrix effect and interferences than can affect sensitivity of OTA detection. Foerster et al. (2021) found low levels of OTA (0.6%, 1.3 ng/mL) in a

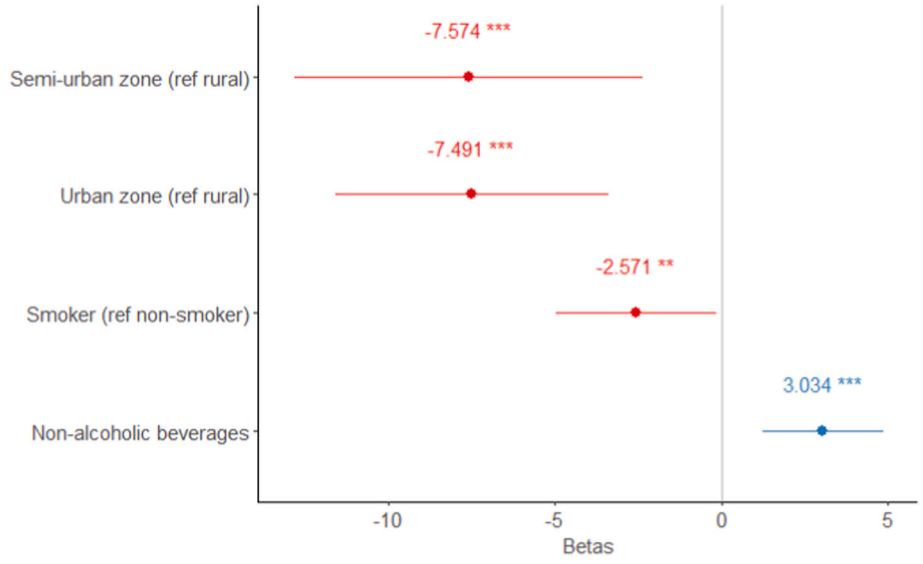

Fig. 1. Multivariate linear regression models with Gaussian distribution results for ENNs concentration (ng/mg) associated factors. Model was adjusted for age and calories intake.

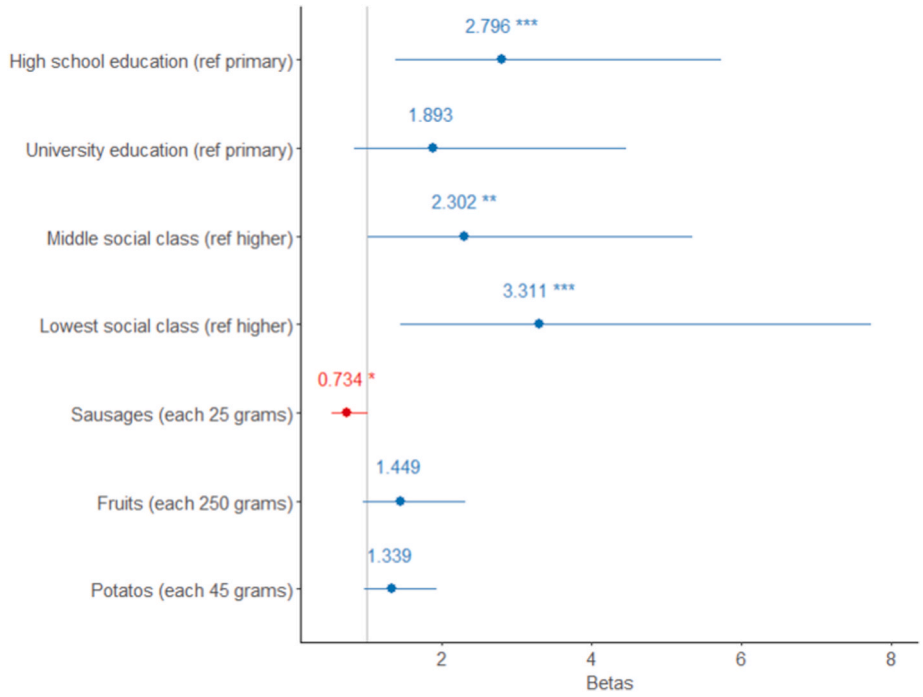

Fig. 2. Multivariate logistic regression models results for presence/absence of mycotoxins' associated factors. Model was adjusted for age and calories intake.

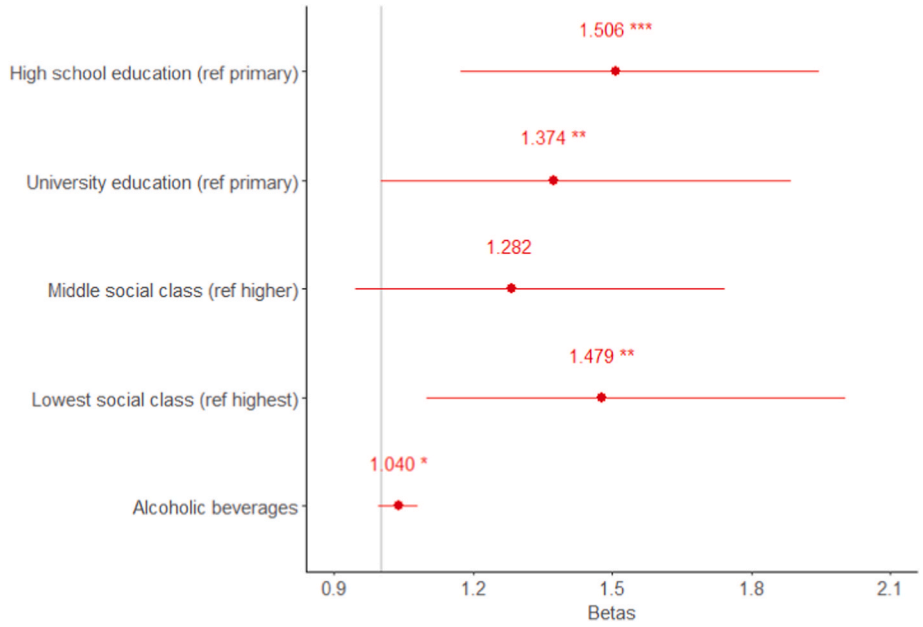

Fig. 3. Multivariate linear regression models with Poisson distribution results for number of detected mycotoxins' associated factors. Model was adjusted for age and calories intake.

rural population of Chile, also using SPE clean-up, so differences in OTA can be related to different levels of food contamination and food habits between countries. Ali et al. (2017) detected OTA in 100% of samples and OTα in 78%, but the metabolite was found in higher levels than the parent mycotoxin, and inter-individual differences in formation and excretion of OTα were observed, which could be also related to dietary differences.

About the results of quantified mycotoxins, in the present study AFs (AFB1, AFB2, AFG1) were quantified in 3% of samples and other metabolites (AFP1, AFM2) were detected in 1% of samples, this low exposure could be expected since these mycotoxins are regulated and are subject of routine food controls. This is in agreement with other authors, which observed a low incidence of AFs and its biomarkers. In similar concentrations than the present study, the previously mentioned study of Foerster et al. (2021) detected AFB1 (8%, 0.3 (±0.3) ng/mL) and AFM1 (2%, 1.8 (±1.0) ng/mL). For a study of a pregnant cohort in rural Bangladesh, low incidence of AFs was reported by Kyei et al. (2022) detecting only AFB2 (<1%, <LOQ) and AFM1 (1.6%, 0.42 ng/mL). Slightly higher exposure was obtained in women urine samples from Nigeria, with 11% of positive samples for AFM1 (Braun et al., 2022).

About ENNs, these emerging *Fusarium* mycotoxins are being further

analyzed, but human exposure data are still scarce. In this study, ENNs were present in 27% of samples, being the third type of mycotoxins most detected. This indicates that their presence in human urine is significant and highlights the need to broaden the knowledge of their harmful effects, to assess whether it is necessary to include them in the legislation and routine food controls. Studies about their occurrence in Italian population, revealed the presence of ENNB in 83.7% (0.006–0.391 ng/mL) and ENNB1 in 94.3% of samples (0.007–0.429 ng/mL), showing a frequent exposure of these *Fusarium* mycotoxins (Rodríguez-Carrasco et al., 2018, 2020). A simultaneous analysis of ENNs and BEA was carried out in Italy, but target mycotoxins were not detected in any sample, which can be explained by different food habits in participants and small sample size (n = 10) (Serrano et al., 2015). Otherwise, in a study carried out in Spain with a sample size of n = 10, presence of ENNs B, B1, and A1 were detected at low concentrations. Four samples were positive for ENB (0.1–0.54 μg L^{-1}), four detectable but not quantifiable (<LOQ) and two non-detectable (<LOD). Regarding ENNB1, two samples reached concentrations of between 0.1 and 0.34 μg L^{-1}, while the other four were below LOQs. Finally, ENNA only detected in one sample in concentrations below LOQ, and BEA was not found in any sample.

4.2. Sociodemographic, dietetic, and environmental factors associations with mycotoxin exposure

In the current study, multivariate analysis for ENNs indicated that living in rural areas was significantly associated with more ENNs exposure, in comparison with urban or semiurban areas. Has been noticed that there are knowledge gaps about mold contamination in rural farming households in low-income settings in Bangladesh, which can lead to a high mycotoxin occurrence in those areas caused by food crops contamination (Kyei et al., 2022).

Smoking showed an inverse association with ENNs exposure, possibly caused by smokers having worse dietary habits and consuming more processed meat instead of fruits, vegetables and whole grains, which are more likely to contain mycotoxins (Dardzińska et al., 2023). Similar results were obtained by Fisher et al. (2016), in which burgers consumers had lower levels of persistent organic pollutants as well as smokers, being also related to dietary habits. Furthermore, regarding exposure to mercury also it was observed that total mercury in blood was lower in women who smoked, and ate less fish (Ramón et al., 2008).

Moreover, tobacco smoke contains lots of oxidants and pro-oxidants species which produce reactive oxygen species (ROS) and reactive nitrogen species (RNS) in the human body, whose excessive amount produces oxidative stress (Kuang et al., 2022). Therefore, as well as some mycotoxins, smoking is a risk factor for cancer, since free radicals present in cigarettes produce oxidative stress, DNA and cell damage and inflammation (Dardzińska et al., 2023).

Nevertheless, although fruits and vegetables can contain more mycotoxins than processed food, usually there is in low levels since most mycotoxins are strictly regulated. Hence their antioxidant effect it is a priority to consider, since has a protective role against mycotoxin and other contaminants damage. Intake of functional ingredients with antioxidant properties as carotenoids, has showed to have a protective role against stress, proinflammatory response and immune toxicity caused by mycotoxins (Cimbalo et al., 2023; Frangiamone et al., 2023).

Moreover, the intake of non-alcoholic beverages was also related with more ENNs exposure, and test with specific food, showed that the most significantly affecting ENNs exposure were light sodas (0.013 [0.001–0.025]) and fruit juice (0.01 [0.0–0.019]). In a study carried out in Spain, 110 alcoholic and non-alcoholic beverage samples were analyzed, resulting in 85% of positive samples, of which beer samples were the ones with higher incidence, followed by wine, cava and cider (Carballo et al., 2021). All these products are obtained from fruits or cereals which are commonly affected by mycotoxin contamination, as showed in a study from China performed in fruits, vegetables and their derivatives as fruit juice, finding that 45.2% of samples were contaminated with *Alternaria* toxins (AME, AOH, TEN, TeA and ALT), caused by mold infection pre and post-harvest (Ji et al., 2023b).

In order to assess ENNB exposure through diet, a study was carried out in Catalonia (Spain) analyzing cereal-based products and ENNB was widely detected, close to 100% in all foodstuffs, co-occurring with deoxynivalenol. Consumption data showed that bread and pasta were the most consumed products in adults (Gallardo et al., 2023). According to this, also a co-occurrence of ENNs and BEA was found in breakfast cereals in Italy, with 93% of samples containing some mycotoxin, which highlights the high contamination incidence of cereal-based products and the exposure to non-regulated mycotoxins (Narváez et al., 2023). Regarding the presence or absence of any detected mycotoxin or metabolite (qualitative), multivariate analysis evidenced that participants with lower social class were associated with higher mycotoxins exposure, as for participants with higher education, agreeing with Fisher et al. (2016) which reported that mothers with higher educational level had higher contaminant concentrations. Regarding food intake, fruits and potatoes showed a positive association, while processed meat as sausages were related with less detection of mycotoxins or metabolites. In food products water activity has shown to have an important role in fungi growth and mycotoxins production as well as temperature conditions, during processing and storage (Romero Donato et al., 2022). The use of NaCl has shown to reduce the growth of mycotoxin-producing fungi in dry-cured meat products, being salting an important part of the processing, as well as water activity and drying and ripening temperatures in order to reduce the production of mycotoxins (Pizzolato Montanha et al., 2018). The use of innovative technologies alongside conventional ones, has proved to be effective to prevent mycotoxin formation and degradation (Gavahian et al., 2020).

Finally, results for multivariate analysis depending on the number of detected mycotoxins or metabolites (quantitative), also showed a positive association with participants with lower social class. Besides, about diet associations, alcoholic beverages were associated with higher mycotoxins or metabolites exposure, and test with specific food indicated that the most significantly affecting was beer. Barley is susceptible to mycotoxin contamination in field or during storage, in a study carried out in Lleida, with beer samples varied by their origin, brewing technology and alcohol content, 20.3% of samples were positive for some mycotoxin, being ZEN the most frequent (65%) and also detected DON, DON-3G, FB1 and HT-2 (Pascari et al., 2018). In the present study alcoholic beverages were associated with higher mycotoxins or metabolites exposure, and test with specific food indicated that the most significantly affecting was beer, which agrees with the widely known contamination of cereal-based products.

Multivariate analysis in the present study evidenced that participants with lower social class showed higher mycotoxins exposure. It is widely known the influence of socioeconomic status with the availability of healthier food option, however a study carried out in Finland highlighted that parental educational level was more associated with children's consumption of fruits and vegetables, than the family income (Serasinghe et al., 2023). Lower socioeconomic status is associated with less access to healthy foods and having more pro-inflammatory diets, which is a risk factor for metabolic health. Wood et al. (2023) studied the association between socioeconomic status and metabolic status during pregnancy, finding that food desert severity was significantly associated with low socioeconomic status.

4.3. Strengths and limitations

There is scarce investigation correlating mycotoxin exposure from biological samples with associated factors. Furthermore, this work includes several parent mycotoxins and metabolites among different families. All this allows to obtain a wider vision of the real exposure to contaminants and to investigate their possible sources of exposure. This study belongs to a large prospective study that includes several Spanish cohorts, in which other contaminants are being studied in different

biological matrices, allowing to possess a large database with different information of the same cohort which can be correlated. The validated quantification method employed in this work allows to detect several mycotoxins and metabolites in complex samples, besides being possible to perform posterior retrospective analysis using the software database. Nevertheless, the study does have some limitations, as only the ten validated mycotoxins were possible to be quantified. Those metabolites detected in a higher percentage could be quantified in the future. About associated factors study, would be interesting to include more variables related to social development in future studies, given the association seen between them.

5. Conclusions

The studied population showed a high exposure to mycotoxins and their metabolites, specially to the non-regulated ENNs, which are related to neurotoxic and genotoxic effects. Higher mycotoxins concentrations were associated with rural areas as well as with participants with lower social class and beer consumers. On the contrary, higher processed meat intake was related to lower mycotoxins' levels. More studies are required to better evaluate the exposure to mycotoxins from food and their environmental relationships, as well as considerate to include more social development related variables which can be confounding factors.

Funding

This research was funded by Generalitat Valenciana [GV/2021/111 and EDGJID/2021/112], Ministry of Universities [CAS21/00008 and NextGeneration EU], Instituto de Salud Carlos III [FIS-FEDER: 13/1944, 16/1288, 17/00663 and 19/1338; FIS-FSE: 17/00260; Miguel Servet-FSE: MSII20/0006].

Author contributions

N.D.-N.: Experimental analysis, Writing—original draft. M.L.: Funding acquisition and Project administration, Writing—review & editing. S.L.: Funding acquisition and Project administration, Writing—review & editing. J.V.: Dietary data acquisition, writing—critical review & editing. A.E.: Participant data acquisition. Writing—review & editing. J.P.: Formal analysis. Writing—review & editing. L.M.: Project administration, Supervision, Writing—review & editing. P.V.-D.: Experimental analysis, Writing—review & editing. J.V.: dietary data acquisition, writing—critical review & editing. All authors have read and agreed to the published version of the manuscript.

Institutional Review Board statement

The study was conducted in accordance with the Declaration of Helsinki, and approved by the Institutional Review Board (or Ethics Committee) of La Fe University and Polytechnic Hospital (4 November 2008).

Informed consent statement

Informed consent was obtained from all participant women involved in the study.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

The data that has been used is confidential.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envres.2023.117618.

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