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ORIGINAL CONTRIBUTION

Plasma ochratoxin A levels, food consumption, and risk biomarkers of a representative sample of men and women from the Molise region in Italy

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Abstract
Background Ochratoxin A (OTA) is a mycotoxin present in food that can be found in human blood, due to its long half-life. Plasma OTA detection represents a good parameter for evaluating the exposure at the population level.
Purpose The relation between plasma OTA levels, dietary habits, and specific disease risk biomarkers (body mass index (BMI), C-reactive protein (CRP), and cardiovascular risk score) was investigated.
Methods The study involved 327 subjects (150 men and 177 women) aged between 38 and 48 years. Food consumption was evaluated by means of the EPIC questionnaire; plasma OTA was measured by HPLC; CRP was determined in fresh serum samples by a latex particle-enhanced immunoturbidimetric assay.
Results OTA was detected in 99.1% of plasma samples (LOD 25 ng/L); the mean ± SD value was 0.229 ± 0.238 ng/mL. However, only 5.2% of samples exceeded 500 ng/L, considered the threshold for a possible pathogenic activity. The estimated mean daily dietary intake of OTA resulted 0.452 ± 0.468 ng/kg body weight (bw)/day, markedly lower than the tolerable daily intake set by EFSA (17.1 ng/kg bw/day). Processed and mutton/lamb meat were found to contribute most to plasma OTA variance. Nevertheless, cereals, wine, beer, and jam/honey consumption correlated positively with OTA levels. Plasma OTA showed a significant positive association with CRP and cardiovascular risk score ($\beta = 0.20 \pm 0.08$; $P = 0.015$ and $\beta = 0.25 \pm 0.08$; $P = 0.001$, respectively); however, the association was present in men but not in women.
Conclusions Even if the hypothesis of a possible hepatic toxicity of OTA in humans is yet to be verified, the positive association between plasma OTA and CRP may indicate a possible role of OTA in inflammation status and consequently in the genesis of cardiovascular diseases and cancer.

Keywords Ochratoxin A · C-reactive protein · Cardiovascular disease · Cancer

Abbreviations

BMI	Body mass index
bw	Body weight
CRP	C-reactive protein
CVD	Cardiovascular disease
OTA	Ochratoxin A
LOD	Limit of detection
LOQ	Limit of quantification
ROS	Reactive oxygen species
RSD	Relative standard deviation
TWI	Tolerable weekly intake

Introduction

Several fungal species of the Penicillium genera (*Penicillium verrucosum*) and Aspergillus genera, section

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Circumdati (*A. ochraceus*, *A. westerdikijae,* and *A. steynii*) and section Nigri (*A. carbonarius* and *A. niger* from *A. niger* aggregate), produce a potent mycotoxin called Ochratoxin A (OTA) [1].

Classified by the International Agency for Research on Cancer (IARC) as class 2B—a possible human carcinogen—[2], OTA shows as well nephrotoxic, immunotoxic, teratogenic and, possibly, neurotoxic and genotoxic properties [3]. Furthermore, it has also been suggested that OTA can alter blood coagulation and can induce histopathological changes in rat heart [4]. Although OTA has been identified as a possible causal agent of human Balkan endemic nephropathy (BEN) [5, 6], its possible involvement in this disease has been considered questionable by FAO/WHO [7].

With regard to diet, it has been shown that consumption of cereals and derived products, dried fruit, coffee, cocoa, some spices, liquorice, wine, grape juice, beer, raw, and pork products indeed increases the human exposure to OTA [8]. In human blood, OTA has a very long half-life (35.5 days) [9], and although renal excretion represents the main elimination mechanism, it seems that OTA can also be excreted, even if at very low amount, with human milk [10]. For instance, a high OTA concentration has been detected in both serum (100% occurrence of positive samples) and mother's milk (73–85% incidence of contaminated samples) Italian samples [11–13].

Thus, considering its long half-life in human blood and its widespread presence in several common foods, plasma OTA detection represents a good biomarker for evaluating the exposure levels for populations [3, 14]. In view of the link between OTA contaminated foods and the occurrence of kidney diseases [10] or oxidative damage [3], in this study, we aimed at identifying possible associations between plasma OTA levels, diet, and markers of disease risk, such as body mass index (BMI), C-reactive protein (CRP), and global individual cardiovascular risk.

Materials and methods

Population for analysis and sampling

A survey on OTA plasma concentration in a representative sample population from the Moli-sani Project [15] in Molise (Southern-Italy region) was carried out by the Research Laboratories of the Fondazione di Ricerca e Cura "Giovanni Paolo II" of Campobasso.

The population for analysis was apparently healthy, since subjects reporting cardiovascular or other major diseases were excluded. Serum (for CRP analysis) and plasma (for OTA analysis) samples were collected from 341 individuals (154 men and 187 women) aged between 38 and 48 years, mean age 41.5 ± 2.6 years; 14 subjects were excluded because of missing values for 1 or more of the eligibility criteria ($n = 327$, 150 men and 177 women). In order to avoid introducing confounding due to an acute inflammatory condition, 16 subjects were further excluded because of serum CRP concentrations > 10 mg/L; therefore, analyses were based on 311 apparently healthy subjects (145 men and 166 women).

Protocol was approved by Ethical Committee of the Catholic University and thus in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki. All participants provided written informed consent.

Dietary assessment

The Italian validated European Prospective Investigation into Cancer and Nutrition Food frequency questionnaire (EPIC FFQ) [16] was used in the survey of participants [16]. The questionnaire consists of 188 food items, grouped into predefined 48 foods/food groups (Appendix). The Nutritional Analysis of Food Frequency Questionnaires software (NAF; National Cancer Institute, Milan, Italy) [17] was used to convert information about dietary data into daily intake of food groups, energy, and macro, and micronutrients.

Anthropometric and other measurements

Body weight and height were measured according to standardized procedures [15].

Based on information collected during a medical interview, smoking status was categorized as "non-smokers," "ex-smokers," and "current smokers"; socio-economic status was defined as a score based on 8 variables, the final score ranged from 0 to 8, while physical activity was defined using 24 questions on working time, leisure time, and sport participation and expressed as daily energy expenditure in metabolic equivalent task-hours (MET-h) [15, 18]. In the present study, the latter three variables were considered as potential confounders.

Definition of cardiovascular disease risk score

The risk equations of the CUORE project, determined on Italian men and women aged 35–69 years without any history of cardiovascular disease (CVD), were applied in order to compute the global individual cardiovascular disease risk [19]. Based on sex, age, systolic blood pressure, total and HDL cholesterol, diabetes, smoking, and use of anti-hypertensive drugs, these equations describe 10-year risk of major fatal and non-fatal cardiovascular events [19].

Biochemical measurements

Fasting blood samples were collected between 07:00 and 09:00 am, and from current smoker participants only if they further refrained from smoking for at least 6 h. High sensitivity CRP (inter- and intra-day coefficients of variation: 5.5 and 4.17%, respectively) was measured by a latex particle-enhanced immunoturbidimetric assay (IL Coagulation Systems on ACL9000).

Analysis for OTA

Reagents

Chemicals and solvents were of HPLC grade or equivalent (Carlo Erba, Milan, Italy). All water used was distilled and, for HPLC, obtained from a Milli-Q purification system (Millipore, London, UK). Acetonitrile and acetic acid used for mobile phases were of HPLC grade and provided by Merck (Darmstadt, Germany). The immunoaffinity columns for OTA were purchased from Vicam (Milford, MA, USA). All the analyses were performed in subdued light. Phosphate buffered saline (PBS) was prepared as per Vicam (NaCl 8 g/L, KCl 0.2 g/L, Na_2HPO_4 1.15 g/L, KH_2PO_4 0.2 g/L; pH 7.4).

Analytical standard

OTA standard was obtained from Sigma-Aldrich (St. Louis, MO, USA). A solution of OTA (40 mg/L in benzene/acetic acid 99+1 v/v) was calibrated spectrophotometrically at 333 nm using the value 5,550 L mol^{-1} cm^{-1} for the molar absorption coefficient [20]. The stock solution was stored at −20 °C when not in use. Working standards were prepared by evaporating an exact volume under a stream of nitrogen and re-dissolving the residue in the HPLC mobile phase. Seven OTA standards between 1 and 50 pg were injected.

OTA extraction

OTA was extracted according to Biasucci [13]; 0.5 mL of distilled water, 0.3 mL of 15% trichloroacetic acid solution, and 2 mL of chloroform were added to 2 mL plasma aliquots; the mixture was vortex mixed for 1 min and allowed to stand for 4 h at room temperature, mixing every 30 min. After centrifugation at 3,000×*g* for 5 min, the chloroform phase was carefully withdrawn and transferred to a test tube. The acidic phase and the compact precipitate layer formed between the two phases were again extracted with 2 mL of chloroform for 1 min on a vortex mixer and then centrifuged. The pooled chloroform extracts were concentrated to 2 mL under a gentle stream of nitrogen and extracted three times with 2 mL each of a 0.13 M $NaHCO_3$ solution.

Subsequently, 6 mL of PBS buffer was added to the pooled aqueous solutions, and the extract was purified through the immunoaffinity column. After column washing with 5 mL of PBS, OTA was eluted into a graduated glass vial with acetonitrile (3 mL). The eluate, concentrated to 0.5 mL under a gentle stream of nitrogen, was brought to 1 mL with acetonitrile: water (25 + 75 v/v) and vortex-mixed for few seconds; the extract was then filtered (HV 0.45 µm filter, Millipore, MA, USA) before HPLC analysis.

OTA detection

Analysis was performed using an HPLC instrument consisting of two PU-1580 chromatographic pumps, an AS-1555 sampling system and a FP-1520 fluorescence detector (Jasco Corporation, Tokyo, Japan); the instrument was controlled by Borwin 1.5 software (Jasco Corporation, Tokyo, Japan). OTA was separated on a Luna Phenyl-Hexyl column (5 µm particle size, 150 × 4.6 mm; Phenomenex, Torrance, CA, USA) with a mobile-phase gradient acetonitrile/water, from 35:65 to 67:33 in 15 min; the flow rate was 1.0 mL/min. The detector was set at $\lambda_{ex} = 333$ nm and $\lambda_{em} = 470$ nm. The injection volume was 100 µl and the OTA retention time was 13.4 min.

Statistical analysis

All analyses were performed using SAS software (version 9.1.3 for Windows, Cary, NC: SAS Institute Inc. 2000–2004). Spearman's rank correlation was used to measure the correlation between the consumption of foods obtained from EPIC FFQ (individually and per group, in grams/day) and OTA levels in plasma. A point biserial correlation analysis was, instead, used to measure the correlation between food consumption and OTA concentrations among subjects with low and high plasma levels ($n = 62$; 10th and 90th percentile, respectively). To investigate the relation between plasma OTA and various dietary factors, a stepwise multiple regression model, with OTA concentrations as dependent variables and two categories of independent variables (dietary and non-dietary covariates), was applied. A multivariable linear regression analysis was used to assess the relation between plasma OTA, C-reactive protein, and cardiovascular risk score. The final model was adjusted for dietary variables associated with OTA—identified in the stepwise regression analysis—together with dietary factors shown in our previous study to be predictors of both serum CRP concentrations and cardiovascular risk score [18]. All dietary covariates were adjusted for total energy intake according

to the residual method, and total energy intake was included in all regression models [21]. Among non-dietary covariates, age, sex, smoking habits (categorical), socioeconomic status (categorical), BMI, and physical activity (categorical) were included in the final multivariable model to control for their possible confounding effects.

Multicollinearity among independent variables was assessed in a linear regression analysis using the variance inflation factor [22]. In order to reduce their positive skewness plasma OTA, CRP concentrations and cardiovascular risk score were transformed into natural logarithms. Values for continuous variables are means ± SD (standard deviation). Interactions between OTA levels and, respectively, age, sex, smoking status, obesity, physical activity, and social status in relation to each dependent variable were tested by including interaction terms in the fully adjusted models. P value < 0.05 was chosen as the level of significance.

OTA intake assessment

The Klaassen equation [10] was applied to estimate (1) the daily dietary intake of the toxin from plasma OTA concentrations and (2) to compare this value with the recommended OTA maximum levels. The daily dietary intake of OTA (K_0, ng/Kg body weight/day) was estimated as follows:

$$K_0 = \mathrm{Cl_p} \times C_\mathrm{p}/A = 1.97 \times C_\mathrm{p}$$

where $\mathrm{Cl_p}$ is the plasma clearance (0.99 mL/Kg body weight/day); C_p is the plasma concentration of OTA (ng/ml); A is the toxin bioavailability, estimated at 50%.

Results

Recoveries, detection, and quantification limit

The calibration curves for OTA showed good linearity ($r^2 > 0.996$). For the recovery experiments, uncontaminated plasma samples were used; plasma aliquots were spiked with OTA at two levels, 200 and 500 ng/L. The average recovery values, obtained from three replicates, were 93.9% (200 ng/L) and 92.5% (500 ng/L). The precision was demonstrated by RSD values always below 6.0%. All results were not corrected for recovery. The limit of detection (LOD) and quantification (LOQ) were defined at those levels resulting in a signal-to-noise ratio of 3 and 10, respectively; the LOD and LOQ values were 25 and 50 ng/L for plasma.

Occurrence of OTA

OTA was detected in 324 (99.1%) plasma samples (54.1% women), a percentage of 62% samples showed an OTA concentration below 200 ng/L, while only in 17 samples (5.2%), a concentration higher than 500 ng/L was observed (Fig. 1).

With regard to positive samples ($n = 324$), OTA concentrations ranged from 29 to 2,918 ng/L; the mean ± SD value of the population was 229 ± 238 ng/L. A significant difference between men and women, adjusted for age, was observed (271 ± 306 vs. 194 ± 150 ng/L, respectively, $P < 0.01$) (Table 1).

Based on Klaassen equation, the estimated daily dietary intake of OTA was 0.452 ± 0.468 ng/kg bw/day (Table 1). For women, this value was 0.383 ± 0.296 ng/kg bw/day, while the estimated intake for men was 0.533 ± 0.603 ng/kg bw/day. Even considering the highest detected OTA value, the corresponding daily dietary intake estimate was 5.75 ng/kg bw/day, markedly lower than the threshold value of 17.1 ng/kg bw/day calculated from the TWI set by EFSA (120 ng/Kg bw/week) [3].

Correlation between food consumption and plasma levels of OTA

As shown in Table 2, Spearman's correlation coefficients between the plasma level of OTA and food consumption were weak ($r \leq 0.21$). When point biserial correlation analysis was performed, in order to calculate correlations between plasma OTA and food consumption among subjects with low and high plasma OTA levels (dichotomous variable; $n = 62$ subjects), correlation coefficients were as follows: rice ($r = 0.30$; $P = 0.017$), bread ($r = 0.31$; $P = 0.015$), processed meat ($r = 0.27$; $P = 0.032$), and ice creams ($r = -0.27$; $P = 0.033$) (data not shown). However, due to the large number of correlations performed, the role of chance in this type of analysis cannot be excluded even if correlations were regarded as being statistically significant ($P < 0.05$).

Foods associated with OTA in plasma

The significant positive dietary determinants of OTA in plasma, after stepwise regression analysis controlled for age, sex, social status, smoking habit, physical activity, and BMI, were processed meat ($P < 0.001$), mutton/lamb ($P = 0.002$), and animal fats (visible fat from meat, ham, and poultry skin; $P = 0.026$). The only significant negative determinant was ice cream ($P = 0.021$). These four variables explained 8.4% of the OTA variance in plasma (data not shown).

Associations between OTA and CRP, BMI and cardiovascular risk score

Significant associations were found between concentrations of OTA (in plasma) and CRP (in serum) in the

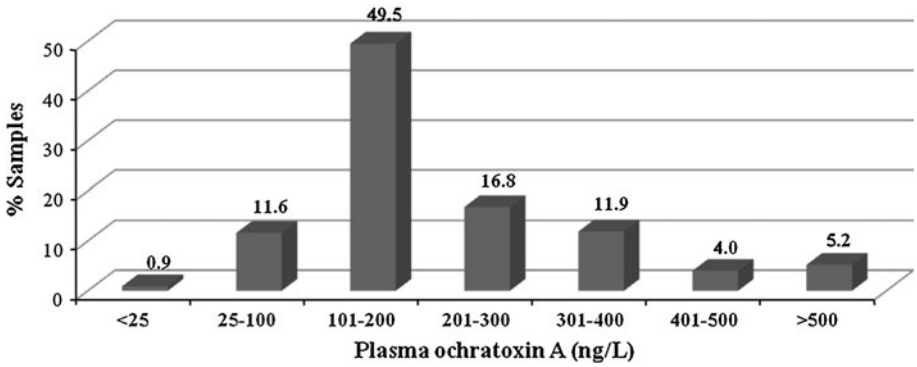

Fig. 1 Distribution of OTA in plasma samples of 324 apparently healthy men and women from the Molise region (Italy)

Table 1 Mean plasma OTA concentrations and estimated daily intake among subjects

Groups	*n*	OTA in plasma (ng/L)		Calculated intake[a] (ng/kg body weight/day)	
		Mean ± SD	Median (IQR)	Mean ± SD	Median (IQR)
All subjects	327	229 ± 238	169 (91–388)	0.452 ± 0.468	0.334 (0.180–0.764)
Women	177	194 ± 150	154 (89–341)	0.383 ± 0.296	0.304 (0.175–0.672)
Men	150	271 ± 306	187 (93–462)	0.533 ± 0.603	0.368 (0.183–0.910)

[a] The daily dietary intake was estimated by applying the Klassen equation [10] see "OTA intake assessment"

Table 2 Correlation coefficients (*r*) between food consumption and plasma levels of OTA among subjects

Food/food groups	Correlation coefficient	*P*
All subjects ($n = 311$)		
Tomatoes (raw, cooked)	−0.12	0.035
Fruits (all types)	−0.12	0.034
Mutton/lamb	0.16	0.005
Visible animal fat	0.14	0.011
Red wine	0.12	0.035
Wine (white, red, rose)	0.13	0.019
Alcoholic beverages (wine, beer, spirits)	0.14	0.012
Women ($n = 166$)		
Yogurt	−0.17	0.029
Honey/jam	0.16	0.035
Rosé wine	0.16	0.041
Men ($n = 145$)		
Tomatoes (raw, cooked)	−0.18	0.034
Fruits (all types)	−0.21	0.010
Mutton/lamb	0.20	0.016
Processed meat	0.19	0.023
Dried fish	0.19	0.021
Fish (all types, canned, dried)	0.18	0.034

Only foods for which the *P* value is significant are listed

whole population, after adjustment for age and sex (*P* for trend = 0.002) (Table 3). In men, OTA was also associated with BMI (*P* for trend = 0.028). However, the latter association became non-significant after adjustment for CRP (*P* for trend = 0.430; data not shown). Interestingly, a significant positive association was also found between OTA levels and cardiovascular risk score (all subjects and men) (Table 3). Adjustment for CRP levels only reduced the strength of the associations in men ($P = 0.041$) but not in the whole population ($P < 0.0001$) (data not shown). In women, no significant associations were found with the variables investigated (Table 3).

The results of multivariable linear regression analyses are shown in Table 4. The fully adjusted models included (1) dietary determinants of OTA (processed meat, mutton/lamb, ice cream, and other animal fat); (2) dietary factors shown in a previous study [13] to be predictors of both CRP and cardiovascular risk score (cooked tomatoes, pasta and other grains, bread, offal, red meat, other dressing sauces for pasta, animal fats, white, red and rosé wine, and beer) and non-dietary covariates included to control for their possible confounding effects (age, sex, and smoking habits—except for cardiovascular risk score—BMI, social status, and physical activity). Thus, a 1% increase in the average OTA value in plasma would yield a 0.20 and 0.25% increase in the average CRP value and cardiovascular risk score, respectively, (Table 4). Sensitivity analysis indicated that the associations were not affected by age, sex, smoking, obesity, physical activity, and social status, all indicators of healthy status, since the interaction terms were not statistically significant (data not shown).

Table 3 Linear regression coefficients of BMI, CRP, and cardiovascular risk score according to log transformed plasma OTA concentrations

Variables	All subjects ($n = 311$)		Men ($n = 145$)		Women ($n = 166$)	
	$\beta \pm$ SEM	P	$\beta \pm$ SEM	P	$\beta \pm$ SEM	P
BMI (kg/m^2)	0.76 ± 0.40	0.060*	1.08 ± 0.49	0.028[§]	0.37 ± 0.66	0.577[§]
CRP (mg/L)[a]	0.27 ± 0.09	0.002*	0.33 ± 0.10	0.002[§]	0.21 ± 0.14	0.137[§]
Cardiovascular risk score[b]	0.38 ± 0.08	<0.0001	0.16 ± 0.06	0.008	0.13 ± 0.07	0.072

* Adjusted for age and sex

[§] Adjusted only for age

[a] Analyses were performed on 311 subjects

[b] Analyses were performed on 301 subjects (142 men and 159 women) because of missing values for cardiovascular risk score

Table 4 Multivariable regression analysis of log transformed CRP and cardiovascular risk score according to log transformed plasma OTA concentrations

Variables	$\beta \pm$ SEM	P
C-reactive protein	0.20 ± 0.08	0.015
CV risk score	0.25 ± 0.08[a]	0.001

Adjusted for age, sex, and smoking habit—except for cardiovascular risk score—BMI, social status, physical activity, processed meat, mutton/lamb, ice cream, cooked tomatoes, pasta and other grains, bread, offals, red meat, other sauces for pasta, animal fats (visible fat from meat, ham and poultry skin), white, red and rosé wine, and beer

[a] Analyses were performed on 301 subjects (142 men and 159 women) because of missing values for cardiovascular risk score

Discussion

OTA levels in plasma

The high incidence of OTA in plasma was in agreement with previous European works [13, 23–25], in which positive percentages higher than 97%, with comparable limits of detection, were found. In similar studies performed in African or Near Eastern countries, these percentages were lower; indeed in Morocco, 60% of samples were found positive to OTA [26], whereas in Lebanon, the percentage decreased to 33% [27], but the detection limits adopted in those works were 80 and 600 ng/L, respectively. In our study, the incidence of OTA level higher than 500 ng/L was 5.2% (LOD 25 ng/L); thus, the Italian OTA exposure results lower than that of the study regarding the Lebanese population.

A higher OTA concentration in plasma of men with respect to women was observed in Italy by Palli et al. [24] and in Switzerland by Zimmerli and Dick [28]. In many other studies cited by Scott [10], no statistically significant difference in OTA levels between sexes was observed. A plasma OTA concentration higher than 500 ng/L was related to the onset of renal diseases [29–31]. From the data reported in Fig. 1, it emerged that 5.2% of samples had a concentration above this threshold and thus a possible pathogenic role of this molecule could regard only a small portion of the population. However, it must be specified that no WHO indication exists about haematic OTA levels, which could be risky for health.

According to Galvano et al. [5], a high haematic OTA level is not a sufficient condition for the development of renal disease. Nevertheless, as reviewed by Scott [10], contrasting results have been reported so far. In particular, it was shown that haematic OTA levels of Tunisian subjects affected by chronic interstitial nephropathy were higher when compared to those of healthy controls or to subjects affected by different types of nephropathy [10]; in contrast, other five studies failed to show any significant difference [10]. In the work of Grosso et al. [29], even if the average OTA concentration in nephropathic patients (990 ng/L, median 540 ng/L) was higher than in healthy subjects (530 ng/L, median 370 ng/L), 19.1% of plasma samples collected from volunteers with renal insufficiency showed an OTA concentration below the quantification level. Moreover, in the same study, 25.8% of healthy subjects showed OTA levels higher than 500 ng/L; this percentage increased to 40.4% in the nephropathic individuals; however, these results did not allow us to establish a clear relationship between OTA levels in plasma and kidney disease. Therefore, the maximum value of 500 ng/L should be regarded as an indicator of a potential harm only. The adoption of the cut-off of 500 ng/L was strengthened also by Hassen et al. [30], who in two different studies found higher OTA concentrations in the blood of subjects affected by nephropathic diseases of unknown origin (mean values, 444 and 504 ng/L), with respect to healthy subjects (26 and 12.2 ng/L).

OTA in plasma and food choice

An important aspect of epidemiological research is to document the dietary determinants of OTA levels in plasma as regards the adult populations.

In the Scientific Cooperation Report "Assessment of dietary intake of OTA by the population of EU Member States (2002)" of the European Scientific Committee on Food (SCF) [32], the mean OTA content in various food products and the mean OTA dietary intake, based on food occurrence and consumption data, have been reported for the different EU member States. This report showed that in Italy, wine was the most contaminated food (1.29–1.49 μg OTA/L), followed by roasted coffee (0.55–1.80 μg OTA/kg), cereals (0.52–0.20 μg OTA/kg), meat, especially pork meat (0.25 μg OTA/kg), and olive oil (0.054 μg OTA/kg). Concerning meat from non-ruminant animals, pork meat is the most susceptible to contamination, due to OTA contamination of pig feeds and the development of molds or the addition of spices in processed meat [5]. On the contrary, meat from ruminants is not usually affected by OTA contamination, since microbes in the rumen degrade OTA to ochratoxin α, a less toxic metabolite [33]. Nevertheless, the report showed also that foods that most contribute to OTA intake are wine (0.86 ng/kg body weight day), coffee, and cereals, at an equal level (0.06 ng/kg body weight day), and pork meat and olive oil (0.01 ng/kg body weight day).

These data fit well with our results for what concerns the correlation between OTA in plasma with bread, rice, wine, and processed pork meat consumption, but not for coffee, probably because of lower consumption levels and the absence of differences in coffee intake in the considered population. Cereals were not identified among the major determinants of plasma OTA variance, probably because consumption is quite consistent in the whole population. The identification of animal fat and mutton/lamb as determinants of OTA levels has never been reported in previous researches. However, we observed a correlation between the consumption of mutton/lamb meat and animal fat with consumption of wine and beer (data not shown); therefore, these results could be explained by the tendency of the population to adopt the habit of consuming specific food items always in the same combination; that is, the consumption of mutton/lamb meat or animal fat in association with wine or beer, which are important OTA sources. A similar situation was also observed by Biasucci et al. [13], who ascribed the association between OTA-free soft drink consumption and higher OTA levels in mother's milk and serum, to the inclusion of soft drinks in a dietary pattern constituting an increased risk of OTA intake. We also observed an inverse correlation between OTA levels in men and in the whole population and tomatoes consumption. Nevertheless, few works considered the contamination of this fruit with OTA, even if Majerus et al. [34] detected OTA slightly above the limit of detection in tomato juices. Both the negative correlation between OTA in plasma with yogurt and fruits and the negative association with ice cream, products that are OTA free, can be explained by the adoption of a diet characterized by an increased consumption of such food products and a corresponding reduction of OTA richer sources, such as cereals, pork meat, and alcoholic beverages. Moreover, a high fruit consumption, besides being correlated to lower OTA levels in plasma, can counteract OTA oxidative effects. In fact, several natural substances of fruit, including tomatoes and vegetables, such as α-tocopherol, carotenoids, and ascorbic acid, show antioxidant properties and may counteract mycotoxin cytotoxicity by blocking mycotoxin-induced free radical damage [35, 36]. Interestingly, Grosse et al. [37] have shown a significant reduction in the levels of DNA adducts if mice treated with OTA were further pretreated with antioxidant vitamins E, A, or C. Furthermore, Baldi et al. [38] have shown that all-trans-retinols are able to decrease OTA-induced ROS production because of their anti-toxic activity in OTA-treated cells.

Associations between OTA in plasma and CRP, BMI, and cardiovascular risk score

Inflammation can be involved in the development of atherosclerosis and cancer; therefore, the relationship between circulating markers of inflammation and OTA levels has been carefully examined. A significant positive association emerged between OTA and CRP, an acute-phase protein, indicating that OTA ingestion could contribute to a systemic inflammatory status of the organism. Although several studies have shown a link between antioxidant (rich) foods and nutrients and CRP levels [39], OTA has never been investigated as positive inductor of CRP synthesis. Nevertheless, our results are consistent with the hypothesis of an OTA-dependent inflammation development, which reflects the toxin cellular effects (lipoxidation, ROS production, and apoptosis) in various organs and tissues, already observed by other authors [40–43].

Considered as nephrotoxic and nephrocarcinogenic in humans [44, 45], OTA was found to induce liver injury in rats, and hepatocellular carcinomas in mice [46, 47]. Even though inflammation plays an important role in cancer development, however, the involvement of CRP is matter of controversial results [48–50]. Potentially considered as a marker of increased cancer risk [49], recently Heikkila et al. [48] further suggested that CRP plays an etiological role in lung, colorectal, and breast cancer. Nevertheless, Allin et al. [50] found no association between elevated CRP levels and increased cancer risk. Finally, the positive

strong association observed between plasma OTA and cardiovascular risk score, independently of other risk factors, indeed represents a new finding that warrants further investigations. Nevertheless, as extensively reviewed by Hussein and Arbid [51], the acute administration of OTA to adult rats produced cardiac disorders and a sequela of direct negative effects on circulatory system. In addition, they reported that OTA compromises both the myocardium and cardiovascular system performances [51]. A possible explanation for why OTA would exert harmful effects on cardiovascular system could be found in an experimental study conducted on rats [4]. Because OTA causes oxidative stress in the heart, the authors investigated whether the administration of OTA in rats might have led to histopathological changes [4]. Indeed, compared to the control group, the authors observed that OTA-treated rats manifested a series of myocardial derangements of different severity [4]. Overall, these and our findings suggest that OTA could play a role also as a possible agent of acquired CVD. However, further studies are needed in order to shed more light on this question.

Conclusions

From this investigation, it emerges that OTA was present in nearly all the analyzed plasma samples, showing a non-negligible average value. The threshold of 500 ng/L of OTA in plasma, which some authors consider an indicator of a possible increased risk for kidney disease [31, 32], has been detected only in 5.2% of the analyzed samples. Positive correlations were found between plasma levels of OTA and the consumption of cereals, processed meat, animal fat, mutton/lamb meat, wine, beer, and jam/honey. For the first time, we found a strong positive association between OTA intake, cardiovascular risk, and CRP.

For these reasons, the hypothesis of a direct involvement of CRP and inflammation, due to OTA intake, in the genesis of CVD and some kinds of cancer, cannot be excluded. Furthermore, in further studies of nephropathies, the presence of OTA in plasma should be considered.

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Conflict of interest The authors declare that they have no conflict of interest.

Appendix

See Table 5.

Table 5 Food items included in each food groups

Foods/food group	Food items
Potatoes	Potatoes
Cooked vegetables	Leafy vegetables, root vegetables, cabbages, onion, carrots, mushrooms, egg plants, artichokes, sweet peppers, spinach, pumpkins, canned vegetables in oil, picked vegetables
Raw vegetables	Raw leafy vegetables, raw tomatoes
Tomatoes	Cooked Tomato sauces, tomatoes
Legumes	Beans, lentils, peas, chick peas
Fruit	Apples, pears, kiwi, bananas, grapes, peaches, apricots, oranges, tangerines, plums, strawberries, melon, khaki, figs, cherries
Nuts and dried fruit	Peanuts, almonds, hazelnuts, walnuts, dried figs, dried dates, prune
Olives	Olives
Milk	Whole, partially skimmed, and skimmed milk
Yogurt	Whole, partially skimmed, and skimmed yogurt
Fresh cheese	Mozzarella, ricotta cheese, taleggio cheese, gorgonzola cheese, melted cheese slices, other soft cream cheese
Seasoned cheese	Fontina cheese, emmenthal, gruyere, parmesan, caciocavallo cheese, other seasoned cheese
Pasta and other grains	Pasta, yellow maize meal
Rice	Rice
Bread	White bread, bread with oil, and other bread
Crisp bread, rusks	Breads sticks, crisp bread
Breakfast cereals	Breakfast cereals
Salty biscuits	Crackers
Red meat	Beef
	Pork
	Mutton/lamb
	Veal
	Other meats (goat game, horse)
White meat	Chicken
	Turkey
	Rabbit
Processed meat	Sausages, ham, bologna sausage, dried beef, salami
Offals	Liver, offals
Canned fish	Canned tuna fish and other fish
Crustaceans, molluscs	Crustaceans, molluscs
Fish	Other fish
Egg	Eggs
Vegetables oils	Seed oils
Olive oil	Olive oil
Butter	Butter
Margarines	Margarines

Table 5 continued

Foods/food group	Food items
Animal fats	Visible fat from meat, poultry skin, fat from ham
Sugar and honey	Sugar and honey
Sweets	Cakes, dry cakes, biscuits, pies, pastries, puddings (non-milk based), brioches
Ice creams	Ice creams
Artificial sweetners	Artificial sweetners
Fruit juices	Orange juice, grapefruit juices, other fruit juices
Soft drinks	Carbonated/soft/isotonic drinks, syrups
Coffee	Coffee
Tea	Tea
Other sauces	Other dressing sauces for pasta
Mayonnaises	Mayonnaises
Soups	Vegetable soup
Bouillon	Meat and stock-cube broth
Snacks	Vegetable quiche
Pizza	Pizza
Wine	Red wine
	Rosé wine
	White wine
Beer	Beer
Spirits	Other alcoholic beverages

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