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Circulating zearalenone and its metabolites differ in women due to body mass index and food intake

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ABSTRACT

The environmental estrogen, zearalenone (ZEA), is found in the food supply from *Fusarium* fungal contamination in grains and sometimes used as a growth promoter for beef cattle. Long-term exposure to ZEA and its metabolites may present health risk due to higher estrogenic activity. Serum ZEA metabolites were measured to determine the exposure and the association with food intake in 48 overweight/obese women (52 ± 9 years). The free and conjugated ZEA indicated the highest detection rate of all the metabolites. Conjugated ZEA and total ZEA metabolites were lower ($p = 0.02$) in overweight/obese than normal weight women, and free metabolites were either the same or showed a trend to be higher. In addition, those with highest (280–480 g/d) compared those with lowest (< 115 g/d) meat consumption had higher conjugated serum ZEA metabolite concentrations ($p < 0.05$). Intakes of other food groups (i.e., dairy, cereal, etc.) were not associated with ZEA metabolites. These findings indicate that ZEA and its metabolites are detectable in nearly all women and concentrations are associated with greater meat intake, and influenced by body mass index. Determining how the food supply influences human concentrations of ZEA metabolites is warranted, as well as determining vulnerable populations.

1. Introduction

Zearalenone (ZEA, also known as ZEN) is a nonsteroidal mycotoxin produced as a secondary metabolite by numerous species of *Fusarium* fungi found across continents, contaminating human foods and grains fed to livestock (Alshannaq and Yu, 2017; Evaluation of certain myc, 2002). Zeranol (α-ZAL), a synthetic derivative of ZEA, is a Food and Drug Administration approved growth promoter for use in beef cattle that is banned in the European Union (Bennett and Klich, 2003). ZEA and its metabolites (ZEA metabolites) have been classified as mycoestrogens, phytoestrogens, and/or growth promoters and are chemically similar to the catechols of endogenous 17-β estradiol and estrone (Bennett and Klich, 2003; Fleck et al., 2012) (See Supplemental Fig. 1). After being ingested with food, ZEA is rapidly absorbed and initially metabolized by the intestine and liver into its major biologically active metabolites, α- and β-zearalenol. In animal models, ZEA exposure has been associated with reproductive dysfunction, cancer and altered immune function, possibly due to its estrogenic activity and the binding affinity of ZEA (and its metabolites) to estrogen receptors (Alshannaq and Yu, 2017; Kuiper-Goodman et al., 1987; Hueza et al., 2014).

However, very few in vivo studies specifically investigating *Fusarium* mycotoxins, including ZEA, have been performed in humans (Escriva et al., 2015). A study examining the relationship between urinary mycoestrogens, breast development, and menarche found measurable levels of ZEA and its associated metabolites in girls, ages 9–10 years (Bandera et al., 2011). Girls having detectable ZEA levels in their urine were found to be shorter and less likely to have reached the onset of breast development (Bandera et al., 2011). Dietary beef and popcorn intake were found to be associated with higher urinary ZEA in the girls. Additionally, a case study of a healthy male, showed that a high compared to no cereal diet, increased urinary ZEA excretion (Warth et al., 2013). Both urinary and serum ZEA have also been reported in pregnant women (Fleck et al., 2016). Other mycotoxin exposure studies in different countries also reported concurrent exposure of multiple mycotoxins in their study populations (Shephard et al., 2013; Wallin et al., 2015; Solfrizzo et al., 2014; Ezekiel et al., 2014). Higher urinary excretion rate of ZEA suggests exposure in persons is attributed to contaminated maize and other grains consumption (Shephard et al., 2013; Ezekiel et al., 2014). In addition, the experimental literature on ZEA exposure indicates potential risk for detrimental health outcomes, yet

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there is limited data in humans. In this study, we aimed to characterize serum free and conjugated ZEA and its metabolites in adult women of various ages and determine whether circulating ZEA metabolites were associated with dietary intake of specific food groups that could contain mycoestrogens. Because both menopausal status and body weight affect estrogenic activity, it was hypothesized that these may also be predictors of ZEA metabolite levels.

2. Methods

2.1. Participants and study design

Fasting serum samples are from the Osteoporosis Weight Loss and Endocrine (OWLE; NIH-AG12161) study, aliquoted and frozen for storage at −70 °C (Pop et al., 2015; Sukumar et al., 2011; Riedt et al., 2005). This cohort study was conducted in healthy, pre- and post-menopausal women (25–69 years of age). Healthy women were recruited at Rutgers University through local newspaper, electronic and radio station advertisements for clinical studies. Participants diagnosed with diseases (i.e. metabolic bone disease, hyperparathyroidism, untreated thyroid disease, significant immune, hepatic, or renal disease, kidney stone in the last 5 years, significant cardiac disease, active malignancy or cancer therapy within the past year) or taking menopausal hormone therapy were excluded, as reported previously (Sukumar et al., 2011; Shapses et al., 2013). Premenopausal women who were not pregnant and postmenopausal women who had not menstruated for at least 2 years were included. Anthropometrics (height, weight, body mass index (BMI)) were measured by balance scale and stadiometer in the clinical laboratory. Certified phlebotomists performed the blood draws. Usual dietary intake was assessed by dietitians on the day of serum sample collections.

The protocols were approved by the Institutional Review Board of Rutgers University (New Brunswick, NJ) and all participants provided written informed consent prior to study procedures. In addition, all participants approved that their samples could be analyzed for questions in future studies.

2.2. Biomarker analysis

Serum samples were transferred to the Chemical Core Analysis Facility of the Occupational and Environmental Health Sciences Institute (EOHSI), Rutgers University. ZEA and its metabolites were measured using LC-MS/MS technique. Total metabolite concentrations were determined with enzymatic deconjugation carried out by adding 10 μL β-glucuronidase from Helix pomatia (Type HP-2, Millipore Sigma, St. Louis, MO) to 0.5 mL serum and 0.25 mL sodium acetate buffer (pH = 4.65) and incubating overnight in a water bath at 37 °C. Free metabolites were measured by adding only the buffer to 0.5 mL serum followed by the incubation. After the two separate analysis for free and total metabolites, the conjugated were estimated from the total minus the free forms. Cleanup for both sample types was performed using a 1 mL ChemElut™ extraction cartridge (Agilent Technologies, Inc., Folsom, CA), eluting the analytes with three 2 ml aliquots of methyl *tert*-butyl ether. The combined eluents were evaporated to dryness and redissolved in 35 μl of 2:1:1 water: methanol: acetonitrile. All analytes were separated and quantitated using a Thermo LTQ mass spectrometer interfaced to an LC system consisting of a Finnigan Surveyor Autosampler plus and a Finnigan Surveyor MS Pump plus. A Hypersil Gold C18, 50 × 2.1 mm, 1.9 μm (Thermo Scientific, San Jose, CA) was used for elution. The solvent gradient was as follows: initial 25% methanol, 50% water, 25% CAN, linear ramp to 35% methanol, 30% water, 35% ACN over 6 min, hold for 4 min, return to the starting composition in 0.01 min, equilibrate for 6 min. The flow rate was 0.2 mL/min. An injection volume of 20 μL was used. Retention times were: 2.30 min for taleranol, 2.46 min for β-zearalenol, 3.10 min for zeranol, 3.31 min for α-zearalenol, 4.04 min for zearalanone, and 4.17 min for ZEA. An atmospheric pressure chemical ionization source was used in negative mode to ionize ZEA and its metabolites before introduction into the mass spectrometer. The precursor ions for the MS method were: m/z 321(zeranol and taleranol), m/z 319 (α- and β-zearalenol, zearalanone) and m/z 317 (ZEA) and the quantitation ions were m/z 277 and m/z 303 (zeranol and taleranol), m/z 275 and m/z 301(α- and β-zearalenol, zearalanone), and m/z 273 and m/z 299 (ZEA). All quality standard control parameters were followed throughout the analysis (Supplemental Fig. 2), including blanks and recoveries for each sample run. Analyte spikes were used for quality control and run with each batch of samples. Recoveries of ± 20% were used to validate the quality of the run. The detection limit for the method was 0.07 ng/mL for all six analytes. When concentrations were below the limit of detection (< LOD) an estimated value was reported based on extrapolation of the curve to zero concentration. The inter-day assay variability (% RSD) was 4.5 for ZEA, 3.2 for α-zearalenol, 2.6 for β-zearalenol, 2.8 for zeranol, 3.0 for zearalanone, and 2.7 for taleranol. The intra-day assay variability (% RSD) was 4.0 for ZEA, 3.1 for α-zearalenol, 2.5 for β-zearalenol, 2.5 for zeranol, 2.8 for zearalanone, and 2.7 for taleranol. Levels of conjugated metabolites were estimated by subtracting the values for free metabolites from the values for total metabolites.

2.3. Food records

Dietary intake was determined using the average of three 24-h dietary recalls, which were conducted by registered dietitians. Dietary intake of food groups was analyzed according to the American Diabetes Association (ADA) exchange groups including meat, grains/cereals, vegetables, fruits, dairy and we also assessed the number of eggs/day and included a bean/tofu category.

2.4. Statistical analyses

Descriptive statistics were used for participants' demographics and to calculate mean, standard deviation, and minimum and maximum values for serum concentrations. One-way ANOVA and Bonferroni post-hoc was used to assess differences between meat groups or BMI category. Due to significant age differences between the different BMI groups, age was included as a covariate in the model (ANCOVA). Also, we tested for normality of the dependent variables using homogeneity of variance testing and as needed, log transformed the ZEA metabolites. Pearson correlation was performed to determine relationships between ZEA metabolites, food intake, weight and BMI. Since food was recorded in ounces as per the ADA exchange system, this unit was used to report values. Multiple regression analysis was used to assess the relative influence of independent variables (BMI, menopausal status and meat intake) on ZEA and ZEA metabolites. Statistical analyses were conducted using the SAS statistical package (SAS Institute, Cary, NC, USA; v 9.4). P value < 0.05 (2-sided) was considered statistically significant and data are presented as means ± SD.

3. Results

Selected characteristics for the women included in the analysis (n = 48) are shown in Table 1. Participants were largely Caucasian (88%) and ranged in age from 25 to 69 years old, with a weight range of 42.7–123.2 kg and BMI range of 18.5–41.3 kg/m^2. Women were categorized by BMI status, and 65% had a normal BMI and 35% were overweight or obese (BMI ≥ 25 kg/m^2).

3.1. Serum concentrations of ZEA and its metabolites

Serum concentrations of ZEA and its metabolites (ZEA metabolites) are shown in Table 2. The serum free ZEA metabolite concentration detection rate ranged from 6.3 to 85.4%. The conjugated concentration