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Urinary mycoestrogens and gestational weight gain in the UPSIDE pregnancy cohort

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Abstract

Background Zearalenone (ZEN), a secondary metabolite of *Fusarium* fungi, is one of the most common mycotoxins in global food supplies such as cereal grains and processed food. ZEN and its metabolites are commonly referred to as mycoestrogens, due to their ability to directly bind nuclear estrogen receptors α (ER-α) and β (ER-β). Zeranol, a synthetic mycoestrogen, is administered to U.S. cattle as a growth promoter. Despite widespread human exposure and ample evidence of adverse reproductive impacts *in vitro* and *in vivo*, there has been little epidemiological research on the health impacts of ZEN exposure during pregnancy. The objective of our study was to examine associations between ZEN and gestational weight gain (GWG).

Methods Urine samples were collected in each trimester from pregnant participants in the UPSIDE cohort ($n = 286$, Rochester, NY, USA). High performance liquid chromatography and high-resolution tandem mass spectrometry were used to quantify concentrations of ZEN as well as ∑mycoestrogens (composite sum of ZEN metabolites; ng/ml). Maternal weights at clinical visits were abstracted from medical records. We fitted longitudinal models of specific-gravity adjusted, log-transformed ZEN and ∑mycoestrogens in relation to total GWG (kilograms) and GWG rate (kilograms/week). We additionally examined risk of excessive GWG (in relation to Institute of Medicine guidelines) and considered effect modification by fetal sex.

Results ZEN and ∑mycoestrogens were detected in > 93% and > 95% of samples, respectively. Mycoestrogen concentrations were positively associated with total GWG (ZEN β:0.50 kg; 95%CI: 0.13, 0.87) and GWG rate (ZEN β:0.20 kg/week; 95%CI: 0.01, 0.03). Associations tended to be stronger among participants carrying male (versus female) fetuses and results were robust to adjustment for diet.

Conclusions Mycoestrogen exposure during pregnancy may contribute to greater GWG. Future research is needed to understand potential influences on downstream maternal and offspring health.

Keywords Zearalenone, Pregnancy, Gestational weight gain, Mycoestrogens, Endocrine disrupting chemicals

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Background

Zearalenone (ZEN) is a secondary metabolite of *Fusarium* fungi and one of the most common mycotoxin contaminants in global food supplies such as cereal grains and processed food [1–3]. Zeranol (ZER), a synthetic version α-zearalanol (a ZEN metabolite), is administered to livestock in the United States to increase rate of weight gain, reducing the time and cost of rearing livestock for the market [4]. ZEN and ZER are commonly referred to as mycoestrogens because their chemical structures are highly similar to 17β-estradiol (E_2) allowing them to bind nuclear estrogen receptors α (ER-α) and β (ER-β) [5]. Some studies examining the underlying mechanism of growth promotion in livestock suggest that ER agonists such as ZER, increase growth hormone concentrations leading to beneficial growth and feed efficiency [6, 7]. Irrespective of the mechanism, there is abundant literature that mycoestrogen exposure not only increases growth in livestock, but also causes impaired reproduction in animal models, including adverse outcomes in offspring following *in utero* exposure [8, 9]. Human biomonitoring studies indicate that exposure to mycoestrogens occurs globally [2], however few studies have examined exposures in pregnant individuals [10, 11]. A recent epidemiological study linked maternal and placental mycoestrogen exposure to altered maternal and cord blood sex steroid hormones [12]. Endocrine disrupting chemicals, such as mycoestrogens, may impact maternal and fetal health outcomes including gestational weight gain (GWG), size at birth, and growth trajectories in both postpartum individuals and their offspring in childhood [13–17]. Monitoring of GWG is part of standard clinical care and is a marker of overall pregnancy health [18]. Excess weight gain (defined by the Institute of Medicine (IOM) as greater than 40, 35, 25, 20 lbs gain for individuals with pre-pregnancy BMI < 18.5, 18.5–24.9, 25-29.9, > 30 respectively) is an important determinant of downstream maternal health, and a risk factor for overweight in infants and high BMI in adolescents [19–22]. Excess GWG, which occurs in roughly 50% of U.S. pregnancies has been linked to macrosomia and caesarean delivery [14, 23]. At the same time, GWG below recommendations (~ 20% of U.S. pregnancies) is associated with small for gestational age and preterm birth [24–26]. Individuals who gain excess weight during pregnancy are at risk for higher postpartum weight retention which raises the risk of cardiometabolic diseases (e.g., type II diabetes, stroke, heart attack) [27–30]. In *in vivo* experiments, administering high doses of mycoestrogens to mice, rats, or swine results in reduced GWG [31–34]. By contrast, in studies of non-pregnant animals, mycoestrogen exposure consistently increases weight gain [4, 35, 36]. Notably the doses in the cited experimental research are often 1000 fold the tolerable daily intake (TDI) for humans of 0.25 μg/kg bw set by the European Food Safety Authority [37]. Most dietary studies report adult exposure below the TDI [38, 39], though some children may exceed the TDI [40].

To the best of our knowledge, the impact of mycoestrogen exposure on GWG has not been studied in humans. Based on the animal evidence in pregnancy, we hypothesized that mycoestrogen exposure would be associated with lower GWG in humans. Using data from a U.S. pregnancy cohort, the objective of this analysis was to examine the relationship between longitudinal urinary mycoestrogen concentrations and GWG, measured continuously as well as in relation to the IOM guidelines.

Methods

Study sample

Pregnant participants ($n = 326$) were recruited into the Understanding Pregnancy Signals and Infant Development (UPSIDE) cohort study at the University of Rochester Medical Center and associated clinics (Rochester, New York, USA) between 2015 and 2019. Informed consent was obtained from all participants. The criteria for enrollment were: (1) at least 18 years of age, (2) early pregnancy (1st trimester), (3) no history of substance abuse, psychosis, or major endocrine disorder, (4) singleton pregnancy, and (5) English speaking [41]. During study visits conducted in each trimester, participants provided biospecimens and completed questionnaires on health, demographics, and lifestyle. Institutional Review Boards (IRB) at the University of Rochester (IRB approval #: 58456, approval date: August 27, 2015) and Rutgers University (IRB approval #: Pro20160001514; January 27, 2017) approved all study activities and all participants provided written informed consent. 286 participants contributed data to the present analysis.

Mycoestrogen concentrations

Maternal spot urine samples were collected in each trimester. A refractometer (Atago 4410 PAL-10S Digital Hand-Held Pocket Urine Specific Gravity Refractometer, Tokyo, Japan) was used to measure urine specific gravity prior to aliquoting and freezing samples at -80° C. Aliquots were sent to the Environmental and Occupational Health Science Institute at Rutgers University (Piscataway, NJ, USA) on dry ice. Urine samples were analyzed for ZEN and metabolites (alpha-zearalenol [α-ZOL], beta-zearalenol [β-ZOL], alpha-zearalanol [α-ZAL/ZER], beta-zearalanol [β-ZAL] and zearalanone [ZAN]) by ultra high-performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS) using previously published methods [12, 42]. An overview of sample preparation and quantitation is provided in Appendix A

(Supplementary Methods). For analytes detected in > 75% of samples, concentrations below the limit of detection were replaced with the value LOD/$\sqrt{2}$ [43]. We applied the Boeniger formula to urine concentrations to adjust for dilution. The formula is Pc (specific gravity corrected analyte concentration) = P[(SGm$_{tri}$-1)/(SG-1)], where P is the mycoestrogen analyte concentration, SGm$_{tri}$ is the trimester median specific gravity for the UPSIDE cohort, and SG is the specific gravity for the sample [44]. Of the 286 participants contributing data to the present analysis, the number of participants who contributed 1, 2, or 3 urine specimens was 14, 26, and 246 respectively.

Gestational weight gain assessment

Weights from all clinical prenatal visits were abstracted from the electronic medical record by trained examiners. As self-reported pre-pregnancy weight may be subject to bias, weight at earliest first trimester clinical visit was used as a proxy for pre-pregnancy weight and was used to calculate pre-pregnancy BMI (kg/m^2). This practice is widely accepted in pregnancy cohorts when clinically recorded pre-pregnancy weight is not available, as first trimester weight gain is typically minimal [45–47].

We calculated trimester specific weight gain, rate of gain in each trimester, total GWG, and rate of gain across pregnancy. Our method for estimating trimester specific weight gain based on all weights in the medical record has been previously described [48]. Briefly, weights at the end of the 1st and 2nd trimester were interpolated based on nearest recorded weights measurements. For participants with gestational weight data recorded within 6 weeks prior to delivery, but no weight within the last week prior to delivery (n = 44), weight on the last day of gestation was imputed [48]. One participant with no weights measured within the final six weeks of pregnancy was excluded from further analysis. Total GWG was calculated as the sum of weight gain in each trimester. Rate of weight gain was the average weight gain across the time period (one trimester, or total). Total GWG was classified as inadequate, appropriate, or excessive based on IOM recommendations for the participants pre-pregnancy BMI (BMI < 18.5 [12.7–18.1 kg], 18.5–24.9 [11.3–15.9 kg], 25-29.9 [6.8-11.3kg], >=30 [5.0-9.1kg]) [18].

Covariates

Potential covariates were selected based on the prior literature and considered using a directed acyclic graph (Supplementary Fig. 1). At enrollment and each prenatal study visit, data on covariates of interest were collected through questionnaires. Additionally, data were abstracted from medical record review. Covariates included: fetal/infant sex, maternal age, education (categorized here as high school or less than high school, some college/college, post-secondary), parity (nulliparous/multiparous), smoking (any or none during pregnancy), gestational age at delivery, and use of social services (any reported use of Women Infant Children Supplemental Nutrition Program, public assistance, or Medicaid during pregnancy versus none). Maternal race and ethnicity may be a proxy for structural racism and injustice that contribute to perinatal health outcomes including GWG, and were therefore included (categorized as Hispanic, non-Hispanic White, non-Hispanic Black, and Asian, mixed race or other race and ethnicity) [49]. Pre-pregnancy BMI, as described in section 2.3, was considered as a continuous variable. In light of prior literature indicating seasonal variation in mycoestrogen exposure, the season of urine collection was categorized as Spring = March, April, May; Summer = June, July, August; Fall = September, October, November, Winter = December, January, February [11]. Additionally, as mycoestrogen concentrations may vary across pregnancy, trimester (1, 2, or 3) was included as a covariate.

We also considered diet as a covariate because exposure is considered to occur through diet for the general population and diet quality along with caloric intake may also influence GWG. Up to three 24-hour dietary recalls per participant were collected in mid-late pregnancy by a trained nutritionist, using the United States Department of Agriculture (USDA)'s Automated Multiple Pass Method [50, 51]. Nutrient intake was calculated by Nutrition Data System for Research software (NDSR, 2017 version, University of Minnesota Nutrition Coordinating Center, Minneapolis, MN) [52]. Based on these recalls we calculated overall energy intake, Healthy Eating Index-2015 (HEI-2015), and percentage of calories from ultra-processed foods (UPF%). The National Cancer Institute method was used to estimate daily energy intake (kcals/day) from diet [53]. HEI-2015, a measurement of overall diet quality, was considered because it is often used to determine how well dietary intake aligns with recommended dietary patterns published in the *Dietary Guidelines for Americans* (*Dietary Guidelines*) [54, 55]. HEI-2015 scores range from 0 to 100 and are the arithmetic sum of nine adequacy and four moderation sub-scales. To calculate UPF%, we adhered to established practices [56]. Specifically, unique food lists compiled from dietary recalls were independently coded by two members of the research team, and differences were resolved by a third member. Composite foods were disaggregated into components and individually coded. UPF% was calculated as (UPF calories/total calories)*100. For participants who provided more than one recall, HEI-2015 and UPF% are averaged across recall days.

Statistical analysis

Based on prior literature, we created a composite measure of total mycoestrogen exposure (Σmycoestrogens) by summing parent and mycoestrogen metabolite concentrations measured in the same sample [57]. Descriptive statistics (mean, standard deviation, percentage, median, and interquartile range) were calculated for sociodemographic, exposure and outcome variables as appropriate. Specific gravity adjusted mycoestrogen concentrations were not normally distributed so log-transformed concentrations were used for all subsequent analyses. Spearman rank correlation was used to examine the relationship between mycoestrogen concentrations, pre-pregnancy BMI, GWG, and dietary parameters. Intraclass correlation (ICC, two-way, mixed effect) was used to assess stability of concentrations across pregnancy.

In the primary analysis, we fitted unadjusted and adjusted longitudinal mixed models for total GWG and average weekly rate of GWG with a fixed effect for mycoestrogens and a random effect for each participant, within an unstructured correlation matrix. Secondarily, we fitted linear regression models to examine mycoestrogen concentrations in individual trimesters in relation to trimester-specific weight gain and total GWG. Final covariate selection was based on a change in the beta estimate for the exposure of > 10%. Covariates retained were maternal race and ethnicity, education, fetal sex, smoking, season of collection, parity, maternal age, pre-pregnancy BMI, and gestational age at delivery. In a sensitivity analysis, we also examined models without adjustment for gestational age at delivery since it is possible mycoestrogens could influence the length of gestation. Given literature on fetal sex influencing perinatal outcomes [23, 58], we additionally considered effect modification by fetal sex in stratified models, and by fitting models with a mycoestrogen*fetal sex interaction term.

In a subset of participants with available dietary information ($n=253$), we examined how the inclusion of dietary parameters might influence associations between urinary mycoestrogens and GWG, by refitting models additionally adjusted for dietary parameters. The models included all covariates utilized in the adjusted models, as well as individually (1) energy intake per day, (2) HEI-2015 score, or (3) UPF%. We also examined fully adjusted models with adjustment for all three dietary parameters.

Finally, using logistic regression models (unadjusted and adjusted), we considered the risk of exceeding IOM GWG recommendations (BMI: <18.5 [12.7–18.1 kg], 18.5–24.9 [11.3–15.9 kg], 25-29.9 [6.8–11.3 kg], >=30 [5.0-9.1 kg] amongst participants who had adequate or excess weight gain ($n=223$). Similar to our primary analysis, we considered models with an interaction term (mycoestrogen*fetal sex) and we conducted a sensitivity analysis without adjustment for gestational age at delivery.

Data analysis was performed in R studio (Version 4.1.0).

Results

Participant characteristics

Participants enrolled in the present study were on average 28.9 ± 4.6 years of age with an early pregnancy BMI of 28.1 ± 4.6 kg/m^2 (Table 1). Most participants were non-Hispanic White (58.0%), completed at least some college (63.3%), and were multiparous (66.1%). More than half of participants (54.2%) report utilizing social services at some point during the index pregnancy. Gestational age at delivery was 39.5 ± 1.5 weeks. Infant birthweight differed by fetal sex (Supplementary Table 1). Study visits occurred at 12.2 ± 1.3, 21.2 ± 1.8, 31.3 ± 1.8 weeks gestation. Participants gained the most weight in the 2nd trimester (6.2 ± 3.2 kg) and average weekly gain peaked in the 3rd trimester (0.5 ± 03 kg/week) (Table 1). 41.3% of participants gained weight above the IOM recommendations. No significant differences in weight gain or IOM guideline adherence were observed by fetal sex ($p=0.23$; Supplementary Table 1).

Mycoestrogen concentrations

In each trimester, ZEN, α-ZOL, and Σmycoestrogens were detected in at least 93.7, 74.9, and 94.8 % of urine samples (Table 2). α-ZAL, ß-ZAL, ß-ZOL, and ZAN were detected in less than 75% of specimens (data not shown) and are not analyzed individually, but are included Σmycoestrogens. Median α-ZOL concentrations trended higher than ZEN concentrations (Supplementary Table 2). Concentrations of ZEN in pregnancies with female fetuses trended higher than in pregnancies with male fetuses but differences were not statistically significant (median males 0.11 ng/ml, females 0.14 ng/ml, p=0.92) (Supplementary Table 1). In the full cohort, mycoestrogen concentrations varied across pregnancy (ICC range 0.18-0.30) (Supplementary Table 2). ZEN concentrations were strongly correlated with α-ZOL and Σmycoestrogens concentrations (Spearman rank r=0.80-0.90, p<0.01) (Table 3).

Bivariate relationships between mycoestrogens, gestational weight gain, and mid/late pregnancy dietary parameters

Maternal pre-pregnancy BMI was inversely related to energy intake (kcal/day), HEI-2015, and total and average GWG (Spearman rank coefficient range: - 0.26 to -0.39, p<0.05), while BMI was positively correlated with percent calories from UPF (r= 0.15, p<0.05) (Table 3). Both energy intake and HEI-2015 were positively correlated

Table 1 Characteristics of UPSIDE study participants contributing data to the present analysis ($n = 286$)[a]

Continuous variables	**Mean (SD)**
Maternal age (years)	28.9 (4.6)
Early pregnancy BMI (kg/m2)	28.1 (4.6)
Gestational age at delivery (weeks)	39.5 (1.5)
Kilocalories per day[b]	2166.8 (317.7)
Percent of calories from UPF[b]	56.0 (17.1)
Healthy Eating Index[b]	53.8 (9.0)
Categorical Variables	**n (%)**
Infant sex (male)	147 (51.4)
Preterm birth (< 37 weeks gestation)	16 (5.6)
Race and ethnicity	
Hispanic	28 (9.8)
Non-Hispanic White	166 (58.0)
Non-Hispanic Black	70 (24.5)
Asian, Pacific Islander, Mixed Race, Other	22 (7.7)
Education	
Less than high school/high school	105 (36.7)
Some college/college	111 (38.8)
More than college	70 (24.5)
Parity (nulliparous)	97 (33.9)
Smoking (any)	21 (7.3)
Use of social service (any)	155 (54.2)
GWG measures	**Mean (SD)**
1st trimester total weight gain (kg)	0.7 (2.3)
1st trimester average weekly (kg/week)	0.1 (0.3)
2nd trimester weight gain (kg)	6.2 (3.2)
2nd trimester average weekly (kg/week)	0.4 (0.2)
3rd trimester weight gain (kg)	5.2 (3.0)
3rd trimester average weekly (kg/week)	0.5 (0.3)
Total GWG (kg)	12.1 (6.2)
Average weekly (kg/week)	0.4 (0.2)
GWG by Institute of Medicine Guidelines	**n(%)**
Below	63 (22.0)
Appropriate	105 (36.7)
Above	118 (41.3)

Abbreviations:*BMI* Body mass index, *UPF* Ultra-processed foods

[a] The n represents participants contributing data in any trimester with the trimester specific n being 1^{st}=271, 2^{nd}=264, 3^{rd}=269

[b] The N for dietary variables is 253 and was derived from 1-3 24-hour dietary recalls in mid-late pregnancy

with total GWG, and UPF% was inversely related to total GWG. ZEN concentrations were negatively correlated with HEI-2015 (r=-0.08, p<0.05), and positively correlated with UPF% and GWG (r=0.09-0.12, p<0.05).

Longitudinal associations between urinary mycoestrogens and GWG

In unadjusted models, ZEN concentrations were associated with greater rate of GWG (β: 0.02 kg/week; 95%CI: 0.01, 0.03) and total GWG (β: 0.57 kg; 95%CI: 0.16, 0.98; Fig. 1, Supplementary Table 3). In unadjusted stratified models, associations tended to be stronger for participants carrying male versus female fetuses. In adjusted models, urinary ZEN concentrations were positively associated with average weekly GWG in all pregnancies (β: 0.02 kg/week; 95%CI: 0.01, 0.03), with no differences observed by fetal sex (male β: 0.02 kg/week; 95%CI: 0.00, 0.04; female β: 0.02 kg/week, 95%CI: 0.02, 0.04). Adjusted associations with total GWG were similarly positive in all pregnancies (β: 0.50 kg; 95%CI: 0.13, 0.87), as well as in mothers carrying male (β: 0.61 kg, 95%CI: 0.09, 1.13) and female fetuses (β: 0.45 kg; 95%CI: -0.06, 0.97). Associations of aZOL and Σmycoestrogens with total GWG were positive, but not statistically significant. Interaction terms for mycoestrogens*fetal sex were not significant (range p-value 0.27–0.93) (Supplementary Table 3). In a sensitivity analysis without adjustment for gestational age at delivery, results were similar to our primary analysis (Supplementary Table 4).

Trimester specific associations between urinary mycoestrogens and GWG

In our secondary analyses, we explored potential trimester-specific windows during which mycoestrogen exposures were most strongly associated with GWG. We observed a consistently positive trend between urinary mycoestrogen concentrations in a given trimester and weight gain in the same trimester (e.g. ZEN: 1st trimester β: 0.24 kg; 95%CI: -0.04, 0.51 ; 2nd trimester β: 0.40 kg; 95%CI: 0.03, 0.75; 3rd trimester β: 0.42 kg; 95%CI: 0.09, 0.75; Supplementary Table 5). Additionally, urinary ZEN concentrations in each individual trimester were positively associated with total GWG (1st trimester β: 0.71 kg; 95%CI: 0.01, 1.41; 2nd trimester β: 0.30 kg; 95%CI: -0.43, 1.03; 3rd trimester β: 0.62 kg; 95%CI: -0.00, 1.25). Associations of aZOL and Σmycoestrogens in relation to GWG measures were mostly positive but non-significant (Supplementary Table 5).

Associations between urinary mycoestrogens and GWG, adjusted for diet

As mycoestrogen exposure is considered to occur exclusively through diet in the general population, and diet influences GWG, we additionally considered models adjusted for dietary parameters. Amongst the subset of participants who had available dietary data from mid/late pregnancy, additional adjustment for diet (energy intake [kcal/day], HEI-2015, and UPF%, or mutually adjusted with all dietary variables) had minimal impact on associations between mycoestrogens and total and/or average weekly GWG. For example, the beta estimate representing the association between ZEN and total GWG without diet adjustment was 0.61 kg; 95%CI 0.21, 1.00, and

Table 2 Distribution of maternal urinary mycoestrogens (ng/ml) across pregnancy UPSIDE cohort ($n = 286$)[a]

Analyte	Timing	N	% > LOD	25%	50%	75%	95%	Max
ZEN	1st	271	94.1	0.057	0.096	0.183	0.642	1.457
aZOL	1st	271	74.9	<LOD	0.078	0.164	0.430	1.506
Σmyco	1st	271	94.8	0.101	0.225	0.460	1.337	4.003
ZEN	2nd	264	99.2	0.064	0.115	0.204	0.528	2.966
aZOL	2nd	264	88.3	0.063	0.132	0.240	0.700	2.621
Σmyco	2nd	264	99.2	0.169	0.319	0.547	1.552	7.661
ZEN	3rd	269	93.7	0.099	0.195	0.345	0.973	10.97
aZOL	3rd	269	90.0	0.122	0.230	0.423	1.092	5.620
Σmyco	3rd	269	97.4	0.262	0.481	0.873	2.421	20.581

Abbreviations: *aZOL* alpha-zearalenol, *Max* Maximum, *Σmyco* Sum of mycoestrogen analytes, *ZEN* Zearalenone

[a] The n represents 286 participants contributing data in any trimester. Mycoestrogen concentrations are adjusted for specific gravity

Table 3 Spearman correlation between log-transformed specific-gravity adjusted urinary mycoestrogen concentrations (ng/ml), dietary parameters, and gestational weight gain ($n = 253$)[a]

	ZEN	aZOL	Σmyco	BMI	Energy intake	HEI-2015	UPF%	GWG rate	Total GWG
ZEN	-	**0.80**	**0.90**	-0.05	-0.04	**-0.08**	**0.12**	**0.09**	**0.10**
aZOL	**0.80**	-	**0.87**	0.06	-0.01	**-0.10**	**0.11**	-0.01	-0.01
Σmyco	**0.90**	**0.87**	-	0.02	-0.01	**-0.15**	**0.14**	0.01	0.02
BMI	-0.05	0.06	0.02	-	**-0.26**	**-0.26**	**0.15**	**-0.39**	**-0.39**
Energy Intake	-0.04	-0.01	-0.01	**-0.26**	-	**-0.10**	0.06	**0.17**	**0.17**
HEI-2015	**-0.08**	**-0.10**	**0.14**	**-0.26**	**-0.10**	-	**-0.38**	**0.19**	**0.18**
UPF%	**0.12**	**0.11**	**0.02**	**0.15**	0.06	**-0.38**	-	**-0.11**	**-0.10**
GWG Rate	**0.09**	-0.01	0.01	**-0.39**	**0.17**	**0.18**	**-0.10**	-	**0.98**
Total GWG	**0.10**	-0.01	0.02	**-0.39**	**0.17**	**0.19**	**-0.11**	**0.98**	-

Abbreviations: *aZOL* alpha-zearalenol, *Energy Intake* kilocalories/day, *HEI-2015* Healthy Eating Index, *GWG rate* Average weekly gain across pregnancy, *UPF%* Ultra-processed foods, *Σmyco* sum of mycoestrogen analytes, *Total GWG* Total gestational weight gain, *ZEN* Zearalenone

[a] The n represents participants who contributed dietary information and mycoestrogen data at least one time during pregnancy. Values below LOD were replaced with LOD/$\sqrt{2}$. Bold indicates significance at $p < 0.05$

after adjustment for diet ZEN estimates ranged from 0.59 to 0.66 kg (Fig. 2, Supplementary Table 6). Additional adjustment for dietary measures did not appreciably change estimates of associations between aZOL or Σmycoestrogens and total and average weekly GWG. Similar to our primary analyses, associations between ZEN and GWG were more strongly positive than associations with aZOL or Σmycoestrogens.

Associations between mycoestrogens and excess weight gain

In adjusted models, we observed non-significantly higher odds of excessive weight gain (versus adequate weight gain) in association with higher mycoestrogen exposure (ZEN: OR 1.13; 95%CI 0.96, 1.34, Σmycoestrogens: OR 1.07; 95%CI 0.93, 1.23; Fig. 3, Supplementary Table 7). The *p*-value for mycoestrogen*fetal sex interaction term in adjusted models was not significant. In models considering only pregnancies with male fetuses, we observed higher odds of excess weight gain (ZEN: OR 1.37; 95%CI 1.05, 1.78, aZOL: OR 1.17; 95% CI 0.91, 1.50; Σmycoestrogens: OR 1.34; 95%CI 1.06, 1.68), but not in pregnancies with female fetuses (ZEN: OR 1.13; 95%CI 0.88, 1.46, aZOL: OR 0.95; 95% CI 0.75, 1.21; Σmycoestrogens: OR 1.02; 95%CI 0.82, 1.28).

Discussion

In this first study on longitudinal mycoestrogen exposure during pregnancy in relation to GWG, we observed that mycoestrogen concentrations across pregnancy were positively associated with total and average weekly weight gain, with strongest associations observed with ZEN exposure. Notably, associations were robust to adjustment for dietary parameters. Additionally, in individuals carrying male, but not female, fetuses mycoestrogen exposures were associated with increased odds of GWG in excess of the IOM recommendations.

Few studies have examined mycoestrogens in pregnant people. In the one prior North American exposure

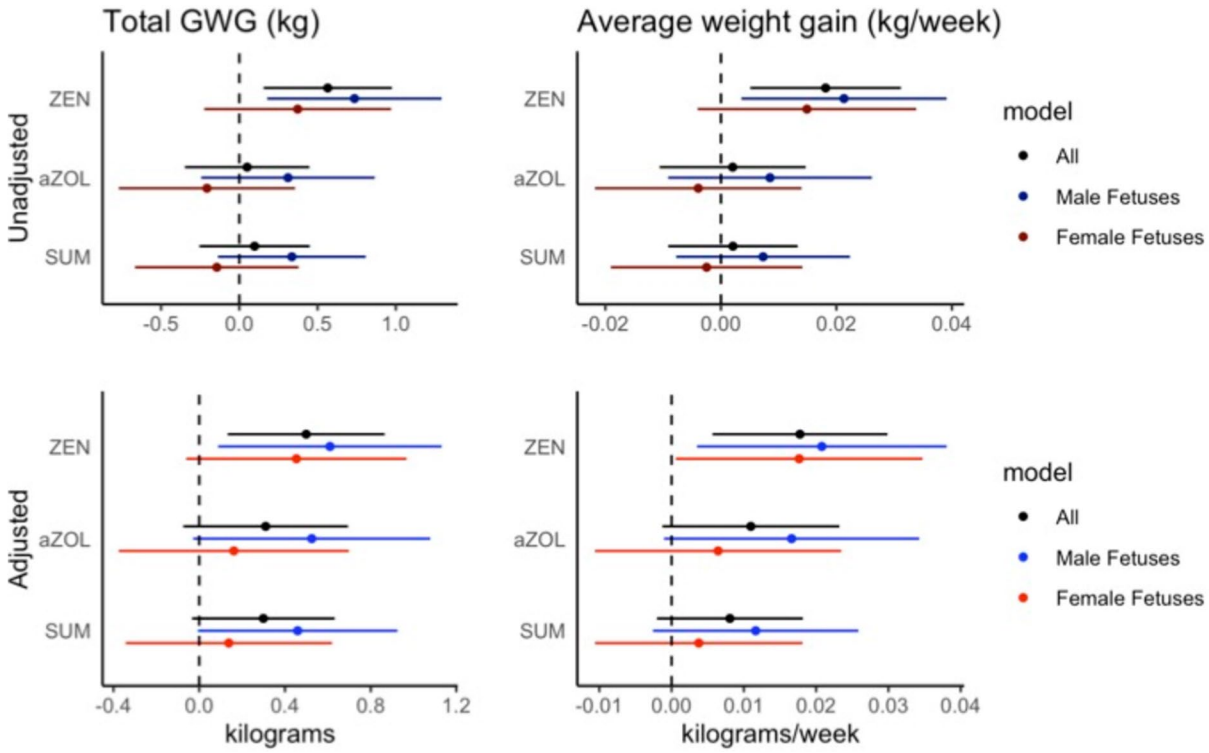

Fig. 1 Longitudinal associations between log-transformed specific-gravity adjusted urinary mycoestrogens and total (kg) and average (kg/week) gestational weight gain in the UPSIDE cohort ($n = 286$). The models are adjusted for maternal age, parity, race/ethnicity, education, pre-pregnancy BMI, fetal sex (in models of all pregnancies only), smoking, season of urine collection, support, gestational age at delivery, and study visit. Values below LOD were replaced with LOD/√2. No interaction was seen for models (p-value for interaction term range 0.27-0.93). Abbreviations: aZOL: alpha-zearalenol, Σmyco: sum of mycoestrogen analytes, Total GWG: total gestational weight gain, ZEN: zearalenone

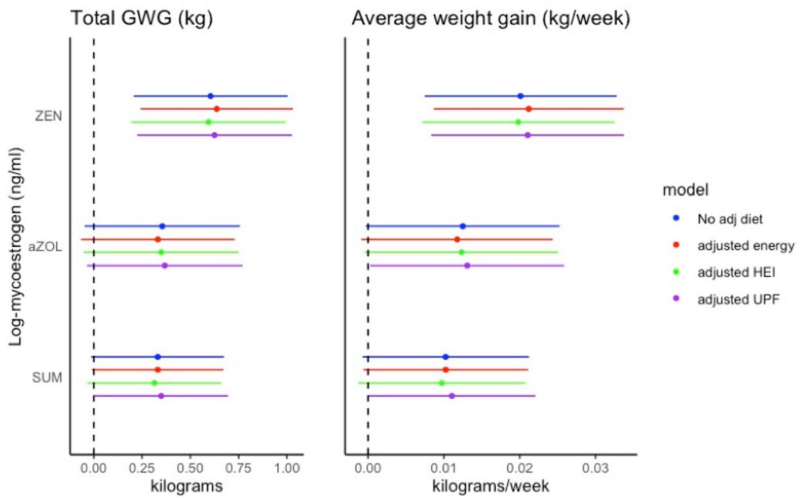

Fig. 2 Longitudinal associations between log-transformed specific-gravity adjusted urinary mycoestrogens (ng/ml) and total (kg) and average rate (kg/week) of gestational weight gain, with additional adjustment for dietary parameters in the UPSIDE cohort ($n = 253$). The dietary parameters are derived from 1 to 3 recalls over the 2nd and 3rd trimester. The 'No Adj Diet' models are adjusted for maternal age, parity, race/ethnicity, education, pre-pregnancy BMI, fetal sex, smoking, season of urine collection, support, gestational age at delivery, and study visit. Each subsequent model is individually adjusted for the same covariates and also (individually) for each dietary parameter (either energy, HEI, or UPF%). Mycoestrogen concentrations below LOD were replaced with LOD/√2. Abbreviations: aZOL: alpha-zearalenol, Energy : kilocalories/day; HEI: Healthy Eating Index, GWG rate: average weekly gain across pregnancy, UPF%: percent of daily calories from ultra-processed foods, Σmyco: sum of mycoestrogen analytes, Total GWG: total gestational weight gain, ZEN: zearalenone

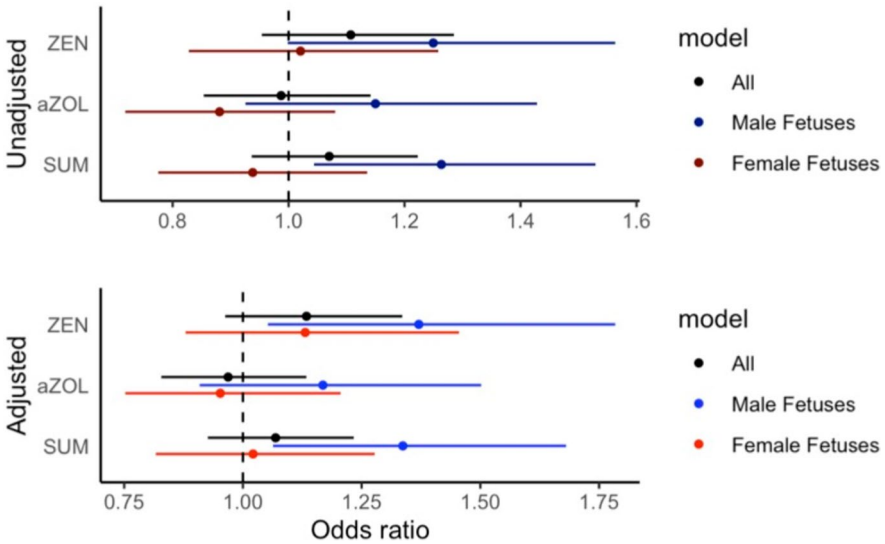

Fig. 3 Logistic regression models examining the odds of gaining weight in excess of Institute of Medicine guidelines (versus appropriate gestational weight gain) in relation to log-transformed, specific-gravity adjusted urinary mycoestrogen concentrations ($n = 223$). The models are adjusted for maternal age, parity, race/ethnicity, education, pre-pregnancy BMI, fetal sex (in models of all pregnancies only), smoking, season of urine collection, support, gestational age at delivery, and study visit. Values below LOD were replaced with LOD/√2. Mycoestrogen concentrations are adjusted for specific gravity. No interaction was seen for all models (p-value for interaction term range 0.29-0.36). Abbreviations: aZOL: alpha-zearalenol, Σmyco: sum of mycoestrogen analytes, ZEN: zearalenone

assessment study, median (0.10 μg/L) ZEN urine concentrations were comparable to those observed in UPSIDE (ZEN median 0.12 ng/ml) [10]. Additionally, a small biomonitoring study of urinary concentrations in Bangladeshi women ($n = 20$) reported mean ZEN (0.057 ± 0.041 ng/ml) and α-ZOL (0.151 ± 0.026 ng/ml), and levels were similar with results reported in the UPSIDE cohort [11]. Ours is the first study examining exposure at multiple time points across pregnancy, and we observed considerable variability in concentrations among study visits (ICC = 0.16–0.22), highlighting the importance of serial assessment for non-persistent chemicals such as ZEN.

Based on experimental studies in mice and rats, our original hypothesis was that mycoestrogen exposure would be associated with reduced maternal weight gain in pregnancy [31, 32]. However in this study, we observed that maternal mycoestrogen exposure was associated with increased GWG. One possible explanation is non-monotonic effects of mycoestrogen exposure, similar to those observed in response to other endocrine disruptors [19, 59, 60]. Of note, the in vivo evidence suggests that higher doses (1-100 mg/kg in mice, 0.3–146 mg/kg in rats per day) may lead to impaired GWG, while lower doses (36 mg ZER implants in 2 month old calves for 100–200 days [approximately 70–130 kg]) may promote weight gain [4]. Differences in response may be due not only to dose, but also species, and unfortunately, at present little is known about human response to mycoestrogen exposure to inform the comparison. Other estrogenic environmental chemicals, including Bisphenol A and certain phthalates, have been associated with patterns of GWG, with differences in magnitude of effect by maternal pre-pregnancy BMI [61, 62].

The mechanism behind modulation of weight gain by mycoestrogens is not known. Four potential mechanisms lead the literature, suggesting that in experimental models, mycoestrogens: (1) are ER agonists thus leading to increases in growth hormone, (2) increase growth hormone independent of ER, (3) impact leptin levels, and (4) modulate glucose transport to increase growth. Notably, there is evidence in humans, sheep, and cattle that an increase in peripheral estrogen concentrations leads to higher serum growth hormone and thus increased growth [63–68]. Studies with exogenous estrogens such as ZER support this hypothesis [6, 7]. In sheep, ZER increased growth hormone as well as insulin-like growth factor-1 (IGF-1), which together support healthy growth of tissue and bones [69]. However, at least one study in cattle concluded that growth hormone concentrations increased following ZER exposure, but that growth outcomes were not associated with growth hormone [70]. Another potential mechanism is mycoestrogens' impact on the weight-regulating hormone leptin, though supporting research is mixed. In one study, sows dosed with ZEN had decreases in serum leptin and reduced backfat [71]. In contrast, ZEN induces leptin secretion in

human breast epithelial cells [72]. These findings, showing divergent impacts of ZEN on leptin, suggest that ZEN may have differential impacts on weight gain and adiposity depending on context. Finally, recent literature has explored how mycoestrogens impact glucose transport. In a murine adipocyte cell line, treatment with ZER led to expression and translocation of GLUT4, as well as increased Akt phosphorylation, facilitating glucose uptake [73]. Further research is warranted as to the mechanisms by which mycoestrogens may impact weight gain (and growth, more generally) in humans, in both the pregnant and non-pregnant state.

We observed that associations between mycoestrogens and total GWG (ß=0.3–0.6 kg; Supplementary Table 6) were robust to adjustment for covariates, including dietary parameters. Additionally, in bivariate analyses GWG was weakly positively associated with HEI-2015 scores ($r=0.19$, $p<0.05$) and weakly inversely correlated with UPF% ($r=-0.11$, $p<0.05$). The association between mycoestrogens and GWG after adjustment suggests that diet quality does not confound this association. We also observed that UPF% was positively correlated with BMI, but not with GWG. This could be because participants with higher BMI are advised to gain less weight per IOM recommendations, or could reflect misreporting of diet.

Our study has strengths and limitations. Utilizing data from the UPSIDE cohort, we were able to adjust for relevant covariates including a range of sociodemographic factors potentially related to exposure as well as to consider the role of diet in these relationships. In the UPSIDE cohort, 41.3% of participants gained excessive weight, which is slightly less than the national estimate (46.5%) [74]. Another strength of this study was our assessment of mycoestrogen exposure at up to three timepoints per participant, which is important given the short biological half-life of ZEN. Finally, we used high quality clinical data and robust analytic approaches to ensure the accuracy of our outcome data on GWG. On the other hand, a limitation of this work is the use of spot urine samples, whereas a pooled 24-hour urine sample (or multiple specimens per trimester) might better capture exposure. Additionally synthetic ZER is structurally identical to the natural ZEN metabolite α-ZAL, therefore we could not specifically measure ZER exposure or assess health endpoints in relation to ZER alone. In this analysis, ZER and α-ZAL, are included in the composite variable Σmycostrogens. As for the outcomes measured in this study, without a clinically recorded weight right before conception, this study relied on weight at the earliest first trimester clinical visits as a proxy for pre-pregnancy weight. Additionally, although diet is believed to be the primary source of exposure, our dietary data was limited to 1–3 recalls per participant in mid-late pregnancy. Ideally, duplicate or triplicate recalls in each trimester could provide more robust data on sources of exposure. According to USDA guidelines and similar to the national HEI average, our population had a mean HEI characterized as "poor diet" [75]. As such, our results may not reflect ZEN exposure in people consuming healthier diets. Finally, the UPSIDE cohort was mostly Non-Hispanic White (58.0%) and data collection was limited to a single U.S. city, therefore the results of this study may not be representative of all U.S. pregnant people.

Conclusions

The potential impacts of mycoestrogens on weight gain in pregnancy present an important public health concern given that mycoestrogens were detected in over 95% of urine samples. These results are relevant to maternal and fetal health, because we connect a known endocrine disrupting chemical (based on animal and in vitro evidence) with an adverse pregnancy outcome (excessive GWG) that has implications for the downstream health of pregnant people as well as their offspring. We demonstrate that mycoestrogens are associated with changes in GWG, a marker of pregnancy health. This is important as mycotoxin exposure is expected to increase in the era of climate change and has been identified as an emerging human health concern by the UN and WHO [3, 76]. Despite an extensive in vitro and in vivo literature of health impacts of mycotoxins, ours is one of the first human pregnancy cohorts to examine this important emerging exposure. To the extent that ZEN exposure may promote excess GWG, ZEN may also contribute to GWG-related adverse birth outcomes such as cesarean section and macrosomia, offspring obesity, and adverse long-term maternal cardiometabolic health [14, 23]. More research is needed to understand the mechanism behind ZEN's impact on GWG, as well as impacts on offspring size at birth and the trajectory of postnatal growth.

Abbreviations

α-ZAL	alpha-zearalanol
α-ZOL	alpha-zearalenol
β-ZAL	beta-zearalanol
β-ZOL	β-zearalenol
BMI	Body mass index
E_2	17β-estradiol
ER-α	Estrogen receptor alpha
ER-β	Estrogen receptor beta
GWG	Gestational weight gain
GWG rate	Average weekly gain across pregnancy
GH	Growth hormone
HEI-2015	Healthy Eating Index 2015
IOM	Institute of Medicine
IGF-1	Insulin-like growth factor
ICC	Intra-class correlation
kcal	kilocalories
LOD	Limit of detection
NDSR	Nutrition Data System for Research
Pc	Specific gravity corrected analyte concentration

SG	Specific gravity for the sample
SGm_{tri}	Trimester median specific gravity for the UPSIDE cohort
Total GWG	Total gestational weight gain
USDA	United States Department of Agriculture
UPLC-MS/MS	Ultra high-performance liquid chromatography-tandem mass spectrometry
UPF	Ultra-processed foods
UPF%	Percent of calories from UPF
UPSIDE	Understanding Pregnancy Signals and Infant Development cohort
∑mycoestrogens	Sum of mycoestrogen analytes
ZAN	Zearalanone
ZEN	Zearalenone

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12940-024-01141-8.

Supplementary Material 1

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Authors' contributions
This study is the result of author contributions as follows: Conceptualization [SG, TO'C, EB, ZR, DF, RM, LA, CK, RB]; Data curation [OW, AB, CK, HS, AK, YM]; Formal analysis [CK, AB, ZR, AK, YM]; Funding acquisition [SG, TO'C, EB, ZR, LA]; Investigation [CK, AB, ZR, EB]; Methodology [BB, AR, ZR, EB, CK]; Project administration [JB]; Resources [BB, EB, ZR, SG, TO'C]; Software; Supervision [EB, BB, ZR]; Validation [CK, AB, BB]; Visualization [CK]; Roles/Writing - original draft [CK]; and Writing - review & editing [All].

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Data availability
The datasets generated and/or analysed during the current study are not publicly available but are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate
This manuscript utilizes data collected from the ongoing prospective Understanding Pregnancy Signals in Development (UPSIDE) birth cohort. Institutional Review Boards (IRB) at the University of Rochester (IRB approval #: 58456, approval date: August 27, 2015) and Rutgers University (IRB approval #: Pro20160001514; January 27, 2017) approved all study activities. Participatns consent was obtained prior to participation in study activities.

Consent for publication
Not applicable.

Competing interests
OW is currently an employee of Exponent, Inc., which provides scientific consulting to the food and beverage industry and reports no conflict of interest. At the time of the data collection and analyses, OW was a student at University of Rochester. Other authors declare no conflicting interests.

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