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Exposure to phytoestrogens *in utero* and age at menarche in a contemporary British cohort☆

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ABSTRACT

Phytoestrogens are estrogenic compounds that occur naturally in plants. Phytoestrogens can cross the placenta, and animal studies have found associations between *in utero* exposure to phytoestrogens and markers of early puberty. We investigated the association between *in utero* exposure to phytoestrogens and early menarche (defined as < 11.5 years of age at onset) using data from a nested case-control study within the Avon Longitudinal Study of Parents and Children, a longitudinal study involving families living in the South West of England. Concentrations of six phytoestrogens were measured in maternal urine samples collected during pregnancy. Logistic regression was used to explore associations between tertiles of phytoestrogen concentrations and menarche status, with adjustment for maternal age at menarche, maternal education, pre-pregnancy body mass index (BMI), child birth order, duration of breastfeeding, and gestational age at sample collection. Among 367 mother-daughter dyads, maternal median (interquartile range) creatinine-corrected concentrations (in μg/g creatinine) were: genistein 62.1 (27.1–160.9), daidzein 184.8 (88.8–383.7), equol 4.3 (2.8–9.0), *O*-desmethylangolensin (*O*-DMA) 13.0 (4.4–34.5), enterodiol 76.1 (39.1–135.8), and enterolactone 911.7 (448.1–1558.0). In analyses comparing those in the highest tertile relative to those in the lowest tertile of *in utero* phytoestrogen exposure, higher enterodiol levels were inversely associated with early menarche (odds ratio (OR)=0.47; 95% confidence interval (CI): 0.26–0.83), while higher *O*-DMA levels were associated with early menarche (OR=1.89; 95% CI: 1.04–3.42). These findings suggest that *in utero* exposure to phytoestrogens may be associated with earlier age at menarche, though the direction of association differs across phytoestrogens.

1. Introduction

Puberty is a crucial period of growth and development. The timing and patterning of pubertal events, such as age at menarche, can provide information on overall health and some previous exposures, while potentially forecasting future health outcomes (Christensen et al., 2011; Biro et al., 2001; Golub et al., 2008).

Age at menarche, on average, has decreased since the late 19th century (Wyshak and Frisch, 1982; Zacharias and Wurtman, 1969), and a secular trend towards earlier development of secondary sexual characteristics has been reported among girls in the Avon Longitudinal Study of Parents and Children (ALSPAC) based in the United Kingdom (Rubin et al., 2009). In the United States, recent estimates for average age at menarche (12.4 years) are almost a year younger than the average age at menarche of women born in the 1920s (13.3 years), and decreases in average age at menarche have been observed across all racial/ethnic groups (McDowell et al., 2007). While improvements in nutritional status since the 19th century and the increasing prevalence of childhood obesity may be in part responsible for this trend, exposure to endocrine disrupting chemicals (EDCs) may also lead to altered timing and patterning of pubertal development (Christensen et al., 2011; Herman-Giddens et al., 1997; Buck Louis et al., 2008; Blanck et al., 2000; Biro et al., 2012).

An EDC, as defined by the U.S. Environmental Protection Agency, is "an exogenous agent that interferes with synthesis, secretion, transport, metabolism, binding action, or elimination of natural blood-borne

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hormones that are present in the body and are responsible for homeostasis, reproduction, and developmental processes" (Diamanti-Kandarakis et al., 2009). EDCs can be natural or man-made, and research suggests that EDCs may have the greatest impact during prenatal and early postnatal development when organ and neural systems are forming (National Institutes of Health, 2015). Most EDCs have estrogenic and/or anti-androgenic actions (Daxenberger et al., 2001), which are thought to have puberty-inducing effects in females (Mouritsen et al., 2010). Previous studies have examined the associations of *in utero* exposure to various EDCs with pubertal development, particularly age at menarche, with some conflicting results (Christensen et al., 2011; Blanck et al., 2000; Vasiliu et al., 2004; Hatch et al., 2011). Most studies were limited by the use of retrospectively-collected age at menarche data.

One potential class of naturally-occurring EDCs of interest is phytoestrogens. Phytoestrogens are estrogenic compounds that occur naturally in plants; two important groups of phytoestrogens are isoflavones (commonly found in legumes like soybeans) and lignans (commonly found in flax seeds, cereal grains, and some fruits) (Kim and Park, 2012; Centers for Disease Control and Prevention, 2008). Although exposure to phytoestrogens is mostly dietary, phytoestrogens can cross the placental barrier in humans (Foster et al., 2002; Todaka et al., 2005).

Phytoestrogens can interact with estrogen receptors, ERα and ERβ, which mediate many downstream activities. The affinities of phytoestrogens for ERα and ERβ are relatively weak compared to estradiol, and the affinities within phytoestrogens for ERα and ERβ vary widely. Additionally, phytoestrogens can have agonist or antagonist activity depending on whether estradiol is also present (Shanle and Xu, 2011; Jefferson et al., 2012; Patisaul and Jefferson, 2010; Cederroth et al., 2012).

Animal studies have reported the effects of phytoestrogens to be quite different according to time, dosage, and route. *In utero* exposure to phytoestrogens are of particular interest because of the timing of differentiation and development (Casanova et al., 1999; Takashima-Sasaki et al., 2006; Takagi et al., 2004). Studies in rodents have found that isoflavones administered through diet or subcutaneous injection during gestation or early life can lead to early vaginal opening (akin to early menarche in humans), irregular estrous cyclicity, and decreased GnRH (gonadotropin-releasing hormone) activation (GnRH coordinates reproductive maturation and function) (Casanova et al., 1999; Takashima-Sasaki et al., 2006; Takagi et al., 2004; Kouki et al., 2003; Lewis et al., 2003; Bateman and Patisaul, 2008; Lee et al., 2009; Nagao et al., 2001). To date, few studies have examined the effects of lignan metabolites on pubertal development, particularly age at menarche (Kim and Park, 2012).

In humans, the effect of soy-based infant formula on pubertal development has been studied to some extent, though this has yielded mixed results regarding an association with age at menarche (Strom et al., 2001; D'Aloisio et al., 2013; Adgent et al., 2011). One retrospective cohort study found no association between soy-based formula and self-reported age at menarche (Strom et al., 2001), while a much larger cohort study that also relied on self-reported recall of age at menarche found that soy-based formula was associated with both very early (≤10 years) and late (≥15 years) menarche (D'Aloisio et al., 2013). Within the ALSPAC prospective cohort study (n=2028), researchers found a 53% increased risk of early menarche among those fed soy-based formula, when compared to cows' milk-based formula (Adgent et al., 2011); it should be noted that this paper examined the ALSPAC cohort as a whole, as opposed to the nested case-control study used in the present study.

There have been no human studies published to date that have investigated the association between *in utero* phytoestrogen exposure and age at menarche. Our aim was to do so, using maternal gestational levels of phytoestrogen exposure and prospectively-collected age at menarche data in a population-based nested case-control study.

2. Study design and methods

2.1. Study population

The Avon Longitudinal Study of Parents and Children (ALSPAC) is an ongoing prospective birth cohort of 14,541 pregnancies. ALSPAC enrolled pregnant women with an expected delivery date between April 1st, 1991 and December 31st, 1992 from three health districts in the former county of Avon, Great Britain. Information has been collected on these parents and children through interviews, mailed questionnaires, and clinic visits. Details on ALSPAC recruitment and study methods have been described elsewhere (Boyd et al., 2013). A nested case-control study was conducted within the ALSPAC cohort to explore associations of prenatal maternal concentrations of various EDCs and age at menarche among the daughters. A 'Growing and Changing' questionnaire was developed to collect information on the offspring's pubertal development and distributed to participants annually between the ages of 8–17 years (1999–2008), with the exception of age 12 (2003). Menarche was determined through parental- or self-report of menarche status, and, if it had occurred, month and year of occurrence so that age could be computed. From the original base population of 14,062 live births, case and control series were selected from singleton (n=11,820) female subjects (n=5756) who had completed at least two puberty staging questionnaires between the ages of 8 and 13 (5 possible questionnaires returned; n=3682). Girls meeting eligibility criteria were ordered according to reported age at menarche when the 13-year old data became available. A cut-off of 11.5 years was established as defining 'early' menarche to satisfy sample size and power needed for the case-control study. Eligible cases could complete any two questionnaires in the series, provided that one was completed after menarche, while controls had to complete the 13-year old questionnaire in order to ascertain that menarche had not occurred by the cut-off of 11.5 years. Of the girls who reported menarche before the age of 11.5 (n=338), 59.8% (n=202) had a prenatal maternal urine sample available, and were considered potential cases. Among girls who reported menarche at or after the age of 11.5, a random sample of 394 was chosen as potential controls, and of these, 61.2% (n=241) had a maternal urine sample available. After evaluating the integrity of the maternal urine samples, 86.1% (n=174) of potential case and 81.3% (n=196) of potential control samples were analyzed. Two cases and one control were excluded due to missing creatinine concentrations, leaving a total sample size of 367 mother-daughter dyads.

2.2. Laboratory analysis

Maternal urine samples were stored at −20 °C before being transferred under controlled conditions to the National Center for Environmental Health, Centers for Disease Control and Prevention (Atlanta, GA) for analysis using high-performance liquid chromatography–tandem mass spectrometry. The analytical methods are described elsewhere (Rybak et al., 2008). Phytoestrogens (genistein, daidzein, equol, *O*-desmethylangolensin (*O*-DMA), enterodiol, and enterolactone) were measured in maternal first morning void urine samples collected at a median gestational age of 12 weeks (interquartile range 8–17 weeks). The isoflavones under study are genistein and daidzein, as well as the daidzein metabolites, equol and *O*-DMA. Also under study are the lignan metabolites, enterodiol and enterolactone, which are metabolites of secoisolariciresinol and matairesinol. Phytoestrogen concentrations were creatinine-corrected using the standardization plus covariate adjustment method of O'Brien et al. (2016). Maternal urine concentrations were used as a proxy for fetal exposure (Green and Marsit, 2015; Ahmed et al., 2011; Engel et al., 2006).