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Impact of gestational electronic cigarette vaping on amino acid signature profile in the pregnant mother and the fetus

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ABSTRACT

Background: Electronic cigarettes (e-cigs) are a form of tobacco product that has become increasingly popular over the past decade. Despite the known health consequences of tobacco product exposure during pregnancy, a substantial number of daily smokers will continue to smoke during pregnancy. Our current knowledge on the effects of e-cig aerosol exposure during pregnancy is limited to a small number of animal studies, which have identified several e-cig aerosol-induced disruptions to the physiology of normal development.

Methods: To further assess the impact of prenatal e-cig aerosol exposure on maternal and fetal health, we examined the amino acid signature profiles in maternal and fetal plasma, as well as in the fetal lungs, a sensitive target organ for prenatal tobacco product exposure. Pregnant Sprague Dawley rats were randomly assigned to one of three groups and were exposed to either e-cig aerosols containing nicotine, e-cig aerosols without nicotine, or room air. Dams were exposed utilizing a state-of-the-art custom engineered e-cig vaping system that is compatible with commercially available e-cig atomizers and enables a translational inhalation delivery method comparable to human vaping.

Results: We determined that gestational exposure to e-cig aerosols results in significant alterations to the amino acid profile in the maternal and fetal compartments, including the fetal lungs. The data shows a targeted disruption to the nitric oxide pathway, branched-chain amino acid metabolism, fetal protein synthesis, and urea cycle.

Conclusion: The data presented herein provides additional support that gestational e-cig aerosol exposure can impact crucial biological processes and exemplifies the need for extensive research on exposure to e-cig aerosols.

1. Introduction

Exposure to tobacco products during pregnancy is known to have a host of detrimental effects on maternal and fetal health, yet an estimated 65% of current U.S. smokers continue to smoke throughout pregnancy [1]. Consumption of electronic cigarettes (e-cigs), one of the latest forms of tobacco products, has increased rapidly over the past decade and has become a popular choice among youth and young adults according to current Center for Disease Control evaluations [2,3]. E-cigs are tobacco products that were originally intended to serve as a cessation and harm reduction tool for traditional cigarette smokers. E-cigs come in a multitude of shapes and sizes, however, all e-cigs operate following a similar set-up, of a battery-powered handheld device that rapidly heats an e-cig liquid (usually containing nicotine and flavorings) to produce an aerosol that is inhaled or "vaped" by the user. In spite of a rise in the popularity of e-cigs, there are few studies examining their effects on human physiology and development. Due to the novelty of e-cig vaping, there are neither long-term studies in humans, nor studies on the effects of e-cigs on human pregnancy. Furthermore, there is only a small set of studies examining the short-term effects of e-cig vaping in adult humans. The limited number of animal studies conducted on the effects of e-cig aerosol exposure during pregnancy have established that e-cigs can negatively affect fetal growth, the cardiopulmonary system, and nervous system development [4–6]. The large knowledge gap on the effects of e-cig vaping necessitates a systematic investigation into how these devices impact pregnancy and development. In order to elucidate potential molecular mechanisms underlying e-cig-induced gestational adaptations, we assessed the concentration of 22 amino acids (AAs) in maternal and fetal compartments that are critical for optimal growth and normal fetal development.

AAs are the basic building blocks for many biological molecules and

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Abbreviations

electronic cigarette (e-cig)
amino acid (AA)
intrauterine growth restriction (IUGR)
pair-fed control (CTRL)
pair-fed group exposed to e-cig aerosols without nicotine (EC-Base)
group exposed to e-cig aerosols containing nicotine (EC-Nic)
gestational day (GD)
branched chain amino acid (BCAA)
nitric oxide (NO)

are involved in a number of functions essential for survival, including protein synthesis/degradation, DNA/RNA synthesis, immune response, and metabolic regulation [7,8]. These compounds are vital to all living organisms, and their concentrations must be maintained to sustain homeostasis [9]. During pregnancy, AAs are transported to the rapidly growing fetus via the placenta [10]. While the fetus is capable of synthesizing some AAs on its own, a large portion of AAs are obtained from maternal circulation [7]. In normal pregnancy, the concentration of AAs in fetal plasma is typically higher than maternal plasma, indicating active transport, but certain pathologies can alter AA transfer across the placenta and consumption of AAs by the fetus [11,12]. Supplementation of specific AAs during pregnancy and lactation has been shown to ameliorate intrauterine growth restriction (IUGR), reduced skeletal muscle mass, and oxidative stress in rats and pigs [13,14]. In humans and animal models, exposure to traditional tobacco smoke results in altered placental morphology that is directly correlated with reduced concentrations of several AAs in maternal and fetal compartments [15,16].

Furthermore, the fetal lungs are an especially sensitive target of prenatal exposure to tobacco products, with strong evidence showing that tobacco exposure in humans can result in reduced respiratory function/capacity, induction of asthma, and development of chronic obstructive pulmonary diseases later in life [17,18]. There is minimal knowledge on the role of AAs in fetal lung development outside the scope of protein synthesis and there are no current examinations on the effects of tobacco products, or nicotine, on the AA profile within the lung. With the invention and rise in the use of e-cigs, the need for investigation has become imperative to assess the effects of prenatal e-cig aerosol exposure on AA concentration in the maternal and fetal plasma, as well as in developing fetal lungs. We hypothesized that prenatal exposure to e-cig aerosols would have a direct impact on the signature profile of the 22 major AAs in maternal and fetal plasma, as well as in male and female fetal lungs of late gestation rats, and thus help identify critical molecular pathways underlying vaping-associated pathologies during development.

2. Methods

2.1. Treatment groups

All experimental procedures were in accordance with National Institutes of Health guidelines (NIH Publication No. 85–23, revised 1996), with approval by the Animal Care and Use Committee at Texas A&M University. Timed pregnant Sprague Dawley rats were purchased from Charles River (Wilmington, MA), and housed in a temperature controlled room at 23 °C with a 12:12-hr light/dark cycle. All rats were breed at 6–8 weeks of age (~200 g body weight; first and only pregnancy). Rats were randomly assigned to one of three treatment groups which included a pair-fed control (CTRL) group exposed to room air; a pair-fed group exposed to e-cig aerosols without nicotine (EC-Base); and a group exposed to e-cig aerosols containing nicotine (EC-Nic). Prior to the start of treatment, dams in CTRL and EC-Base groups were yoked to a dam of similar weight in the EC-Nic group. Diet administered to both pair-fed groups was matched to the daily amount of feed consumed by dams in the corresponding EC-Nic group to control for nutritional effects of e-cig vaping on pregnancy. Pair-fed treatment groups have been previously shown to adequately control for nutritional intake in a prenatal exposure model [19]. In addition to being a nutritional control, the CTRL group served as a control for exposure to e-cig aerosols and for the overall vaping treatment procedure. During the exposure paradigm, CTRL dams were placed in e-cig vaping chambers identical to the chambers used for e-cig vaping treatment. CTRL dams were maintained in these chambers for the same time duration as EC-Base and EC-Nic groups, with only room air flowing through the chamber. The EC-Base group allowed for the identification of differential effects due to e-cig aerosol exposure in the absence of nicotine, however, the majority of human e-cig consumers use vaping devices that contain nicotine. To account for this, the EC-Nic group reflects a physiologically relevant e-cig aerosol exposure with nicotine.

2.2. Vaping treatment

The E-cig vaping treatment was conducted using a custom engineered e-cig aerosol exposure system that allowed for the simultaneous and discreet delivery of either e-cig aerosols or room air to specific chambers as previously described [20]. The binge e-cig vaping paradigm utilized in this study has been shown to produce serum nicotine levels (median peak serum nicotine concentration equal to 27.7 ng/mL), comparable to moderate/high level human smokers and resembles human e-cig vaping topography [20–23]. Additionally, the chemical constituents of the aerosols produced by the vaping chamber system were found to resemble the chemical profile of aerosols derived from human e-cig vaping devices [20,24,25]. Dams were exposed to the vaping treatment for 3 h per day, 5 days per week from gestation day (GD) 5–20 [5,20,26]. Each episode of vaping treatment utilized a commercially available e-cig atomizer (Sense Herakles) that produced a 1 s puff of ~42 mL every 20 s. The e-cig base liquid utilized for the EC-Base group was compounded in-lab with a 80:20 composition ratio of propylene glycol (Fischer) and glycerol (Fischer), respectively. E-cig liquid utilized for the EC-Nic group maintained the same proportional guidelines as the base liquid with the addition of either 5% (50 mg/mL) nicotine during acclimatization or 10% (100 mg/mL) nicotine. During an acclimatization period from GD 5–8, the EC-Nic dams were exposed to e-cig aerosols produced using the 5% nicotine e-cig liquid. Following the acclimatization period, the EC-Nic dams were exposed to e-cig aerosols produced using the 10% nicotine e-cig liquid for the remainder of the exposure paradigm.

2.3. Tissue collection

All groups were sacrificed on GD 21, one day after the last vaping treatment. This study did not contain a humane endpoint prior to date of termination. Growth parameters were collected at the time of euthanasia: maternal weight, fetal weight, fetal crown rump length, and placental weight. Placental weight was used to calculate placental efficiency, as the ratio of fetal body weight to placental weight. Maternal and fetal blood samples were also collected at the time of euthanasia. Dams were quickly euthanized and a hysterectomy was performed to remove the fetuses. Growth parameters were recorded for all fetuses prior to removal of whole lungs from one male and one female per dam. All tissue samples were flash frozen in liquid nitrogen and stored at −80 °C until further processing.

2.4. AA analysis

The AA profiles of maternal and fetal plasma samples were

determined by HPLC analysis following standard procedures [27,28]. Briefly, 0.5 mL of sample was added to a 12 × 75 mm polypropylene tube and mixed via vortex with 0.5 mL of 1.5 M $HClO_4$ and 0.25 mL of 2 M K_2CO_3. After centrifugation of the tube at 3000 g for 15 min the supernatant was collected and used for HPLC analysis. For the determination of AA profile in fetal lung tissues, a portion of tissue (~100 mg) was homogenized in 1 mL of 2 M $HClO_4$ (perchloric acid) and rinsed with 1 mL HPLC-grade water. The homogenate was neutralized with 0.5 mL of 2 M K_2CO_3. The whole solution was centrifuged at 3000 g for 10 min, and the supernatant fluid was analyzed for free AAs using HPLC methods [27,29]. Concentrations of AAs in samples were quantified based on authentic standards from Sigma Chemicals (St. Louis, MO, USA), using the Waters Millennium-32 workstation [30,31].

2.5. Calculations

The unit of analysis was equal to the dam or litter for each group. All groups had n = 6 dams for a total of 18 animals. All animals were included in the analysis. Threshold for statistical significance was determined *a priori* as $P < 0.05$. Maternal weight, fetal weight, fetal crown rump length, and placental efficiency were analyzed using one-way ANOVA with treatment group as the independent variable. Concentrations of individual AAs in the maternal plasma, fetal plasma, and fetal lungs were also analyzed using one-way ANOVA with treatment group as the independent variable.

3. Results

3.1. Growth parameters

Pregnancy related growth measures are depicted in Fig. 1. Maternal weight on GD 21 was not significantly different among the three treatment groups. Fetal weight in the EC-Nic group was found to be significantly decreased ($P < 0.0001$) compared to both the CTRL (↓36.7%) and EC-Base (↓35.4%) groups. Fetal crown rump length in the EC-Nic group was significantly decreased ($P < 0.0001$) compared to both CTRL (↓16.6%) and EC-Base (↓15.4%) groups. Placental weight (not shown) in EC-Nic group was significantly decreased ($P = 0.0014$) compared to both CTRL (↓35.6%) and EC-Base (↓31.6%) groups, however, placental efficiency (fetal weight/placental weight) was not significantly different among treatment groups.

3.2. AA concentrations

Concentrations of AA, in maternal and fetal plasma were determined to examine the amount of free AAs present in the plasma during pregnancy in both maternal and fetal circulation (Fig. 2 and Fig. 3, respectively). Concentrations of individual AAs are listed in supplemental data (Tables 1 and 2 - Supplemental Information). The concentrations of AAs in maternal plasma of the EC-Base and CTRL groups were not significantly different. The maternal plasma of the EC-Nic group showed a

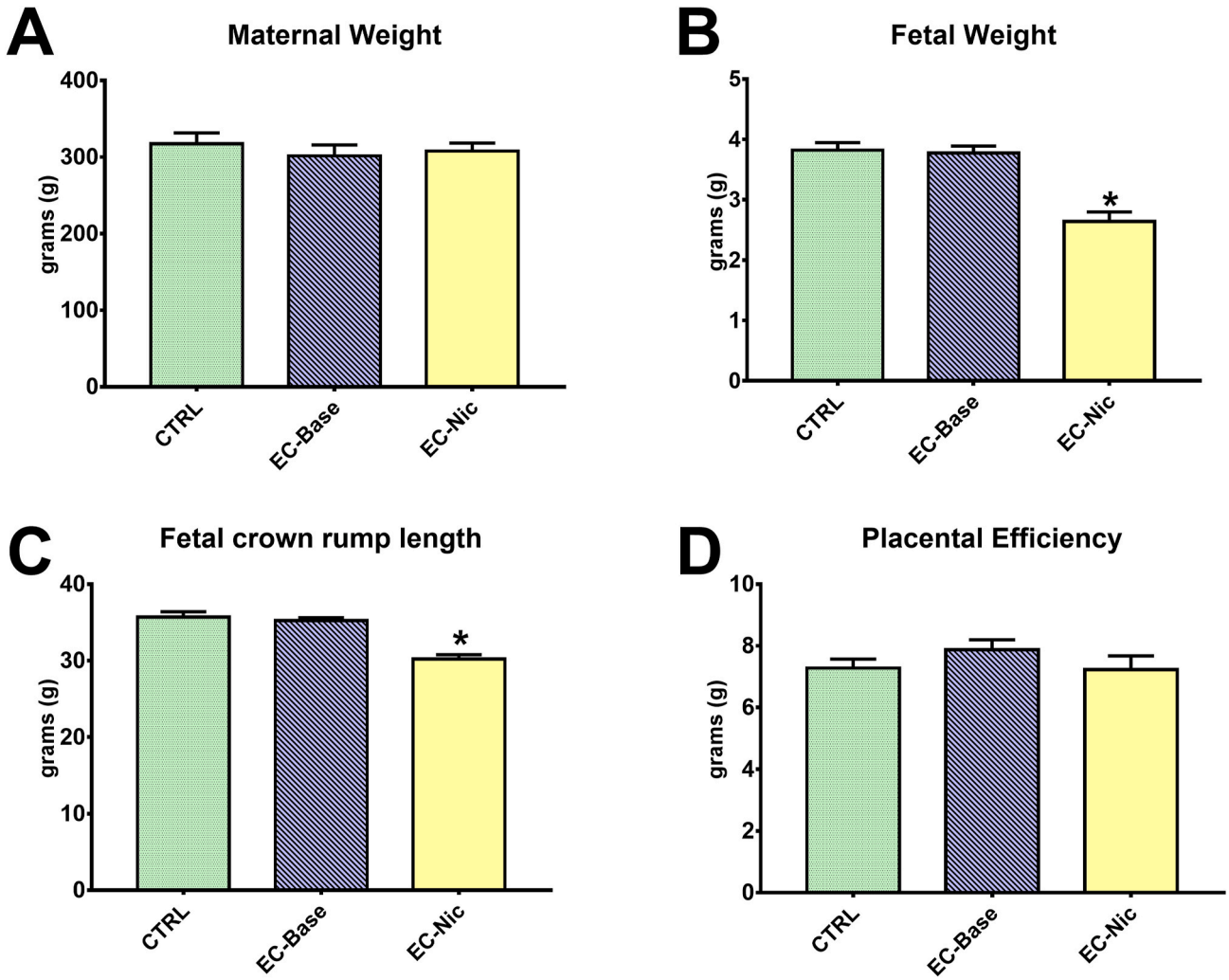

Fig. 1. Effect of prenatal e-cig aerosol exposure on maternal and fetal growth on gestational day 21. Placental efficiency was calculated as ratio of placental weight to fetal weight. *Indicates significant difference compared to Control; $P < 0.05$.

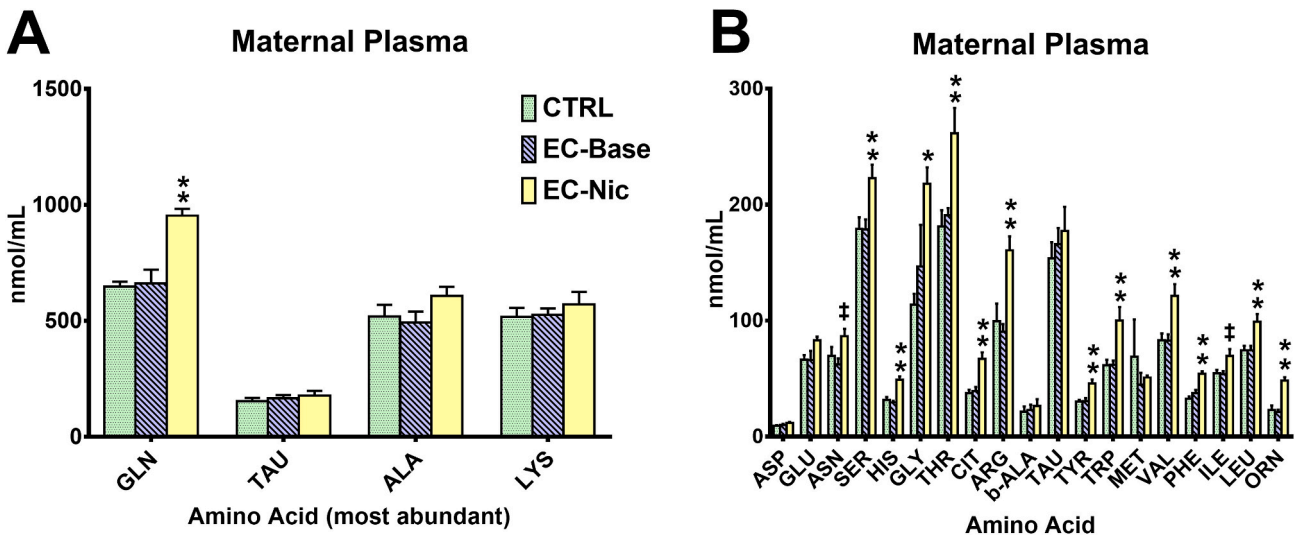

Fig. 2. Effect of prenatal e-cig aerosol exposure on maternal plasma amino acid (AA) concentrations. The AAs altered in maternal plasma of EC-Nic group compared to CTRL are: serine (↑), glutamine (↑), histidine (↑), glycine (↑), threonine (↑), citrulline (↑), arginine (↑), tyrosine (↑), tryptophan (↑), valine (↑), phenylalanine (↑), leucine (↑), and ornithine (↑). The AAs altered in maternal plasma of EC-Nic group compared to EC-Base group are: asparagine (↑), serine (↑), glutamine (↑), histidine (↑), threonine (↑), citrulline (↑), arginine (↑), tyrosine (↑), tryptophan (↑), valine (↑), phenylalanine (↑), isoleucine (↑), leucine (↑), and ornithine (↑). The concentration of AAs in maternal plasma of the EC-Base and CTRL groups were not significantly different. *Indicates significant difference compared to Control; **Indicates significant difference compared to Control and EC-Base; ‡Indicates significant difference compared to EC-Base only; $P < 0.05$.

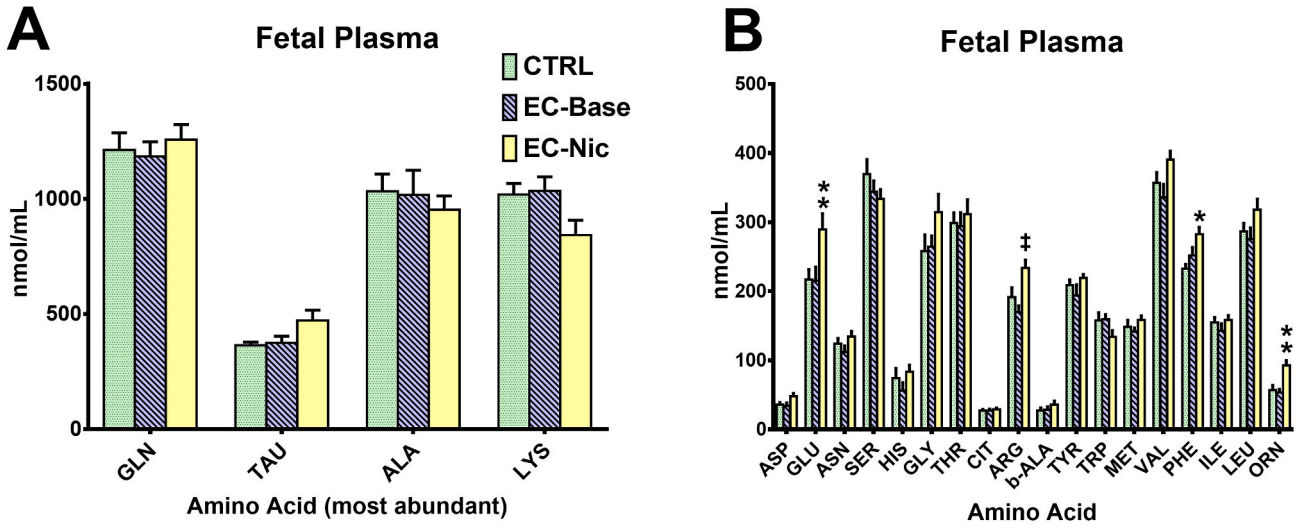

Fig. 3. Effect of prenatal e-cig aerosol exposure on fetal plasma amino acid (AA) concentrations. The AAs altered in the fetal plasma of the EC-Nic group compared to CTRL are: glutamate (↑), phenylalanine (↑), and ornithine (↑). The AAs altered in the fetal plasma of EC-Nic group compared to EC-Base group are: glutamine (↑), arginine (↑), and ornithine (↑). The concentration of AAs in fetal plasma of the EC-Base and CTRL groups were not significantly different. *Indicates significant difference compared to Control; **Indicates significant difference compared to Control and EC-Base; ‡Indicates significant difference compared to EC-Base only; $P < 0.05$.

significant increase in the concentration of 13 AAs compared to those in the CTRL group (Fig. 2). The AAs altered in maternal plasma of the EC-Nic group compared to CTRL were the following: serine ($P = 0.0201$), glutamine ($P = 0.0002$), histidine ($P = 0.0002$), glycine ($P = 0.0144$), threonine ($P = 0.0062$), citrulline ($P = 0.0005$), arginine ($P = 0.0064$), tyrosine ($P = 0.0027$), tryptophan ($P = 0.0071$), valine ($P = 0.0063$), phenylalanine ($P < 0.0001$), leucine ($P = 0.0083$), and ornithine ($P < 0.0001$). The maternal plasma of the EC-Nic group also showed significant differences in the concentrations of 14 AAs compared to those in the EC-Base group (Fig. 2). The AAs altered in maternal plasma of the EC-Nic group compared to EC-Base group were the following: asparagine ($P = 0.0459$), serine ($P = 0.0197$), glutamine ($P = 0.0003$), histidine ($P = 0.0001$), threonine ($P = 0.0151$), citrulline ($P = 0.0008$), arginine ($P = 0.0022$), tyrosine ($P = 0.0035$), tryptophan ($P = 0.0071$), valine ($P = 0.0088$), phenylalanine ($P = 0.0004$), isoleucine ($P = 0.0426$), leucine ($P = 0.0086$), and ornithine ($P = 0.0001$). The concentrations of AAs in the fetal plasma of the EC-Base and CTRL groups were not significantly different. The fetal plasma of the EC-Nic group showed a significant increase in the concentration of three AAs compared to the CTRL group (Fig. 3). The AAs altered in the fetal plasma of the EC-Nic group compared to CTRL were the following: glutamate ($P = 0.0421$), phenylalanine ($P = 0.0066$), and ornithine ($P = 0.0039$). The fetal plasma of EC-Nic group also showed significant differences in the concentration of three AAs compared to those in the EC-Base group. The AAs altered in the fetal plasma of the EC-Nic group compared to EC-Base group were the following: glutamine ($P = 0.0372$), arginine ($P =$

0.0038), and ornithine (P = 0.0018).

Concentrations of AA, in male and female fetal lungs from each group, were compared to determine the accumulation or deficit of AAs in tissues targeted by prenatal tobacco product exposure and to establish sex-linked effects of e-cig aerosols on the developing respiratory system (Fig. 4 and Fig. 5). Concentrations of individual AAs are listed in supplemental data (Tables 3 and 4 - Supplemental Information). The male fetal lungs of EC-Nic group showed a significant increase in the concentration of 11 AAs compared to those in the CTRL group. The AAs altered in the male fetal lungs of EC-Nic group compared to CTRL were the following: aspartate (P = 0.0226), glutamate (P = 0.0016), asparagine (P = 0.0071), threonine (P = 0.0018), citrulline (P = 0.0142), arginine (P = 0.0114), methionine (P = 0.0456), valine (P = 0.0019), isoleucine (P = 0.0074), leucine (P = 0.0167), and ornithine (P = 0.0007). The only AA found to be significantly different in male fetal lungs of EC-Nic compared to EC-Base group was ornithine (P = 0.0003). The male fetal lungs of EC-Base group showed a significant difference in the concentration of threonine (P = 0.0144) compared to CTRL group, whereas concentrations of glutamate (P = 0.0560), citrulline (P = 0.0999), arginine (P = 0.0755), and valine (P = 0.0605) trended to be different compared to CTRL group. The signature of AAs that were impacted in the male and female fetal lungs of the EC-Nic group compared to CTRL shared a number of similarities. The female fetal lungs of EC-Nic group showed a significant difference in the concentration of 9 AAs compared to the CTRL group. The AAs altered in the female fetal lungs of EC-Nic group compared to those in the CTRL were the following: aspartate (P = 0.0455), glutamate (P = 0.0045), asparagine (P = 0.0052), glutamine (P = 0.0395), threonine (P = 0.0047), citrulline (P = 0.0403), valine (P = 0.0088), isoleucine (P = 0.0355), and ornithine (P = 0.0021). The only AA found to be significantly different in female fetal lungs of EC-Nic group compared to EC-Base group was ornithine (P = 0.0098). The female fetal lungs of EC-Base group showed a significant difference in the concentration of threonine (P = 0.0145) and alanine (P = 0.0449) compared to those in the CTRL group, whereas the concentrations of citrulline (P = 0.0956) and arginine (P = 0.0964) trended to be different compared to those in the CTRL group. A similar pattern in the identity of dysregulated AAs in the male and female fetal lungs suggests that there are minimal sex-linked effects contributing to prenatal e-cig aerosol induced alterations to AA signature profile, in the developing lungs.

4. Discussion

AAs play an integral role in a number of physiological processes, including regulation of oxidative stress, cell signaling, protein synthesis, acid-base balance, and synthesis of small molecules such as nitric oxide [7,8]. The present study examined AA concentrations in maternal and fetal plasma as well as fetal lung tissue using HPLC analyses to determine the impact of prenatal e-cig aerosols on the AA signature profile during late pregnancy. To our knowledge, the data presented herein are the first to show the impact of prenatal e-cig aerosol exposure on AA concentrations in the maternal and fetal compartments, and is the first study to examine the effects of tobacco products on the AA profile of the developing fetal lung. These novel findings reveal valuable information pertaining to the effects of e-cig aerosol vaping: 1) exposure to e-cig aerosols with nicotine during pregnancy alters the AA profile in maternal and fetal plasma, but e-cig aerosols without nicotine do not; 2) e-cig aerosols with and without nicotine alter the AA profile in both male and female fetal lungs; 3) sex has a minimal effect on the pattern of dysregulation of AAs in the fetal lungs; 4) exposure to e-cig aerosols containing nicotine increased the concentration of ornithine in all major tissues that were analyzed; and 5) patterns of AA dysregulation in fetal lungs of the EC-Nic group may indicate altered nitric oxide production, induced by e-cig aerosol exposure.

Exposure to tobacco products and nicotine during pregnancy is known to produce IUGR in human and animal models [32,33]. Rodent models of prenatal nicotine exposure have been critical to understanding the altered physiology of pregnancy as it relates to human development. While no animal model is perfectly analogous to humans, pregnancy induced vascular adaptations and pulmonary development are well established in rodent models [34–36]. We previously demonstrated that our model of prenatal exposure to e-cig aerosols containing nicotine produces significantly reduced fetal and postnatal growth, which is accompanied by a reduction in blood flow in the maternal uterine artery and fetal umbilical artery [20]. Of the 22 AAs measured in this study, the concentrations of more than half were found to be dysregulated in the plasma of dams exposed to e-cig aerosols containing nicotine, when compared to EC-Base and CTRL groups. Nicotine appears to be the main influencing factor on AA signature profile alterations in the mother, since there were no significant differences between the EC-Base and CTRL maternal plasma. Analyses of fetal plasma revealed a

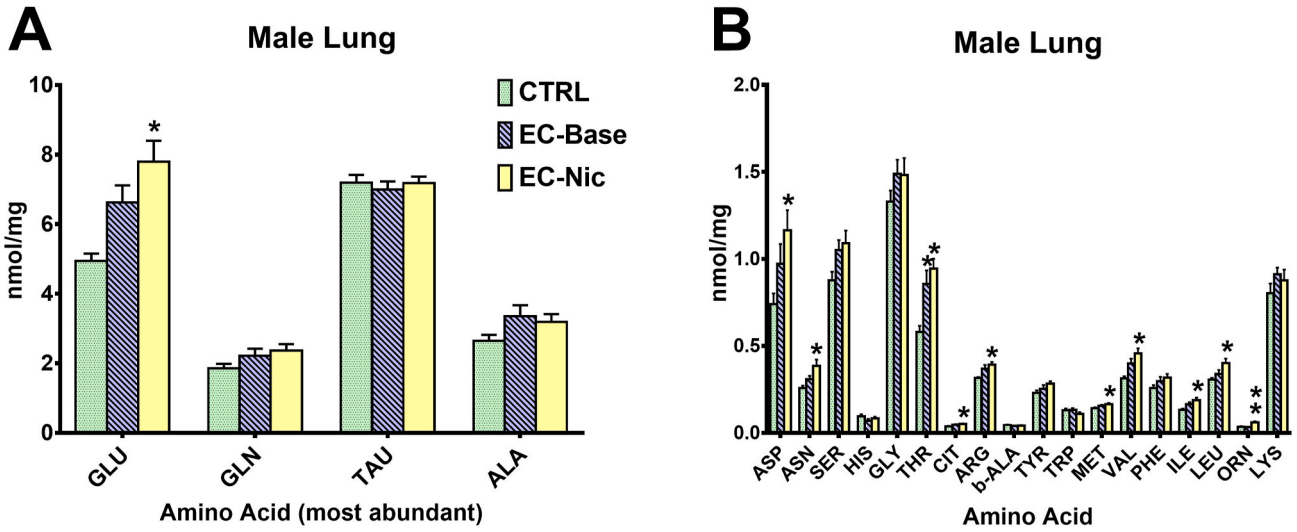

Fig. 4. Effect of prenatal e-cig aerosol exposure on male fetal lung amino acid (AA) concentrations. The AAs altered in the male fetal lungs of EC-Nic group compared to CTRL are: aspartate (↑), glutamate (↑), asparagine (↑), threonine (↑), citrulline (↑), arginine (↑), methionine (↑), valine (↑), isoleucine (↑), leucine (↑), and ornithine (↑). The only AA found to be significantly different in male fetal lungs of EC-Nic compared to EC-Base group was ornithine (↑). The male fetal lungs of EC-Base group showed a significant difference in the concentration of threonine (↑) compared to CTRL group. *Indicates significant difference compared to Control; **Indicates significant difference compared to Control and EC-Base; P < 0.05.

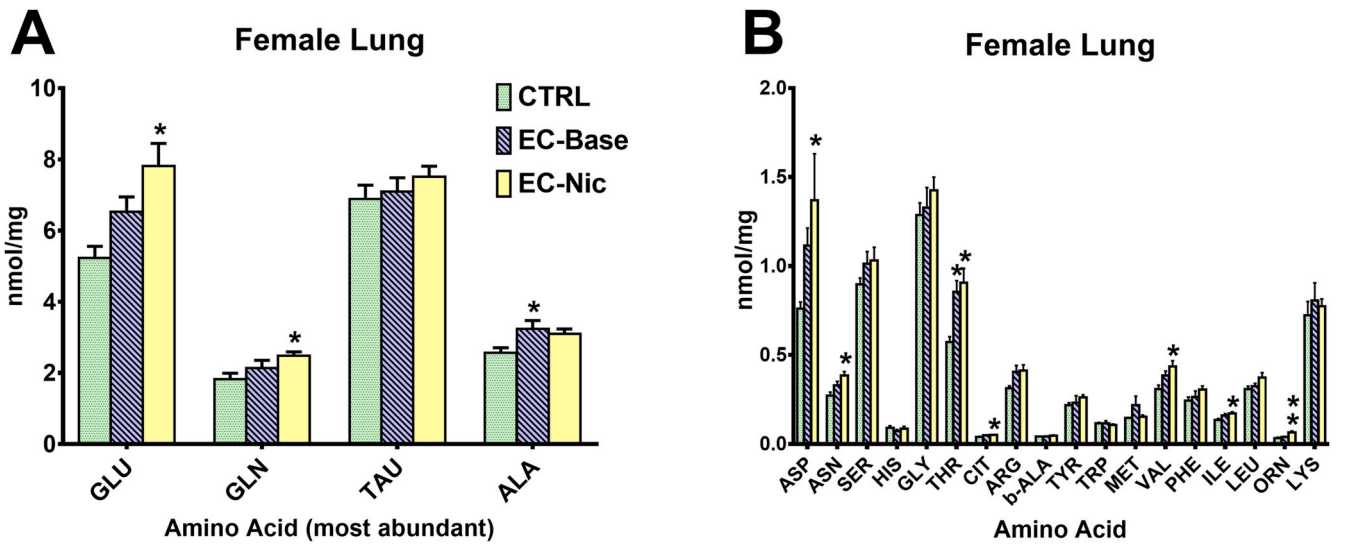

Fig. 5. Effect of prenatal e-cig aerosol exposure on female fetal lung amino acid (AA) concentrations. The AAs altered in the female fetal lungs of EC-Nic group compared to CTRL are: aspartate (↑), glutamate (↑), asparagine (↑), glutamine (↑), threonine (↑), citrulline (↑), valine (↑), isoleucine (↑), and ornithine (↑). The only AA found to be significantly different in female fetal lungs of EC-Nic compared to EC-Base group was ornithine (↑). The female fetal lungs of EC-Base group showed a significant difference in the concentration of threonine (↑) and alanine (↑) compared to CTRL group. *Indicates significant difference compared to Control; **Indicates significant difference compared to Control and EC-Base; $P < 0.05$.

smaller number (three) of AAs that were dysregulated by e-cig aerosol exposure with nicotine. Similar to maternal plasma, there were no differences in AA concentrations in fetal plasma between EC-Base and CTRL groups. In normal pregnancy, there is a significant correlation between maternal and fetal plasma AA concentrations [37]. Early studies in humans have shown that IUGR is correlated to a significant increase in the concentration of maternal plasma AAs, yet fetal plasma concentrations are reduced; these studies may partially explain the alterations reported herein [12,38]. In contrast, we found that the fetal plasma of EC-Nic group showed a significant increase in the concentration of several AAs that may be attributed to a decreased catabolism of amino acids (especially glycine), a decrease in protein synthesis, an increase in protein degradation, or their combination, leading to reduced fetal protein synthesis in a growth-restricted fetus. An increase in the circulating level of glycine (a precursor of glutathione [a major antioxidant peptide]) may be an adaptation response of the dam to oxidative stress.

The rate of AA transport across the placental barrier is determined by hormonal regulation, solute concentration gradients, and the abundance and availability of binding sites of specific transport proteins within the placental tissue [39–41]. Although these transport proteins were not quantified in our model, nicotine is known to reduce the transfer of AAs across the placenta by inhibiting active and facilitated transport [42–44]. Thus, despite a significant increase in the concentrations of maternal plasma AAs, many of these increases may not be reflected in fetal plasma due, in part, to decreased transport across the placenta and/or decreased fetal protein synthesis. The combined effects of IUGR and placental exposure to nicotine may potentially produce a pattern of increases in AA concentration in the maternal plasma that does not directly correlate to the pattern of AA concentration dysregulation expressed in the fetal plasma.

Previous studies on the effects of tobacco products and nicotine exposure during pregnancy has labeled the fetal lungs as a susceptible target of developmental dysregulation, which may result in lifelong complications such as asthma and the development of chronic obstructive pulmonary disease [45–47]. There is very little data examining the AA profile of fetal lungs, and there are no current evaluations on the effects of prenatal e-cig aerosol exposure on fetal lung AAs. In this study, exposure to e-cig aerosols with and without nicotine had a significant effect on the concentrations of several key groups of AAs in the fetal lungs. In fetal lungs exposed to e-cig aerosols with nicotine, there was significant dysregulation in 11 of the 22 AAs in males and 9 of the 22 AAs in females, with nearly complete overlap in the identity of altered AAs between the two sexes. In male fetal lungs, the concentrations of arginine, methionine, and leucine were significantly different in the EC-Nic group compared to CTRL, but were not different in the female lungs. In the female lungs of EC-Nic group, the concentration of glutamine was significantly different compared to CTRL, but was not different in male fetal lungs. The only AA to be altered in both male and female lungs of EC-Nic and EC-Base groups compared to CTRL was threonine. In the female lungs of EC-Base group, there was also a significant difference in the concentration of alanine compared to CTRL. In the EC-Base group there was a trend towards significant difference in the concentrations of glutamine, citrulline, arginine, and valine in the male fetal lungs, and a trend towards significant difference in citrulline and arginine in the female fetal lungs compared to CTRL group. Patterns of AA dysregulation in the male and female fetal lungs of the EC-Nic group does not suggest that sex plays a major role in the dysregulation of the AA profile in fetal lungs exposed to prenatal e-cig aerosols. An increase in the concentration of branched-chain amino acids (BCAA – valine, leucine, and isoleucine) in the fetal lungs of the EC-Nic group may indicate protein degradation, insulin resistance, and a potential source of inflammation [48,49]. *In vitro* studies that examined the effects of the presence of exogenous BCAA on mouse endothelial cells, proposed that BCAA results in the activation of mTORC1 which modulates the production of reactive oxygen species, inflammatory gene expression, and leukocyte adhesion [50]. Importantly, there were significant differences in AA concentrations in both the EC-Nic and EC-Base group, which indicates that chemical constituents other than nicotine in the e-cig aerosols do have an effect on the lungs and may contribute to altered fetal development supporting the claim that fetal lungs are susceptible to developmental dysregulation induced by prenatal exposure to tobacco products like e-cigs.

Exposure to e-cig aerosols containing nicotine may also contribute to altered pulmonary development by disrupting nitric oxide (NO) production. NO is a major signaling molecule that contributes to a large number of physiological pathways and is known to mediate several aspects of pulmonary development [51,52]. In fetal rat lung explants, branching morphogenesis of airways was increased by the addition of a NO donor up to a certain concentration, with higher concentrations of

NO resulting in diminished airway branching, demonstrating a need for strict NO regulation in fetal lung development [53]. NO is generated through the conversion of arginine to citrulline, catalyzed by nitric oxide synthase (NOS) [54,55]. Previous studies have shown that the supplementation of citrulline in neonatal rats, and subsequent increase in NO production, ameliorates reduced alveolar growth and pulmonary hypertension in a model of O_2-induced bronchopulmonary dysplasia [56]. NOS activity can be inhibited by the presence of arginase II, which is responsible for the conversion of arginine to ornithine in the urea cycle and competes for arginine as a substrate [57]. Incidentally, ornithine was the only AA that was significantly increased in all tissue types of the EC-Nic group compared to EC-Base and CTRL groups. Increased concentrations of citrulline, aspartate, arginine, and ornithine in the fetal lungs may indicate an e-cig-induced redirecting of arginine from the NO synthesis pathway to the urea cycle. The sequestering of arginine supply from the NO synthesis pathway to the urea cycle, as documented in several experiments and cell types, is accompanied by reduced NO production [58,59]. Without sufficient NO, the fetal lungs may not be able to develop normally and may result in prenatal e-cig aerosol-induced respiratory pathologies in neonatal and adult life.

4.1. Perspectives

The data herein are novel for offering a glimpse into the relevant molecular alterations potentially contributing to prenatal e-cig aerosol-induced disruption to pregnancy, in both the mother and the fetus. This study was limited to the analysis of free AAs in the tissues examined, therefore, future investigations are needed to expand on the mechanisms underlying e-cig aerosol-induced alterations to the AA signature profile during pregnancy. AAs are the base unit of proteins and are crucial for a number of biological pathways, especially during pregnancy. Although e-cigs are used as a harm-reduction tool for traditional tobacco smokers, there is growing evidence that e-cig aerosols with and without nicotine can have damaging effects on the physiology of pregnancy and development. The data obtained from this study provides additional support that gestational e-cig aerosol exposure can impact crucial biological processes and exemplifies the need for extensive research on exposure to e-cig aerosols during pregnancy.

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CRediT authorship contribution statement

Marcus R. Orzabal: Methodology, Investigation, Formal analysis, Visualization, Writing – original draft, Writing – review & editing. **Vishal D. Naik:** Investigation, Visualization, Writing – review & editing. **Jehoon Lee:** Investigation, Visualization, Writing – review & editing. **Guoyao Wu:** Methodology, Investigation, Writing – review & editing. **Jayanth Ramadoss:** Conceptualization, Funding acquisition, Project administration, Writing – review & editing.

Declaration of competing interest

The authors have no relevant financial or non-financial interests to declare.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.metop.2021.100107.

Availability of data

All data generated or analyzed during this study are included in this published article (and its supplementary information files).

Code availability

Not Applicable.

Ethics approval

All experimental procedures were in accordance with National Institutes of Health guidelines (NIH Publication No. 85–23, revised 1996), with approval by the Animal Care and Use Committee at Texas A&M University.

Consent to participate

Not Applicable.

Consent for publication

Not Applicable.

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