Association of Early Pubertal Onset in Female Rats with Inhalation of Lavender Oil

Yoo-Mi Kim (ym.kim@cnu.ac.kr)
Chungnam National University School of Medicine https://orcid.org/0000-0002-8440-5069
Han H. Lim
Chungnam National University Hospital

Research

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Abstract

Background

Central precocious puberty is caused by early activation of the hypothalamic–pituitary–gonadal axis but its major cause remains unclear. Studies have indicated an association between chronic environmental exposure to endocrine-disrupting chemicals and pubertal onset.

Methods

To evaluate this association, we compared the hormone levels and timing of vaginal opening in female rats exposed to lavender oil (LO) through different routes [study groups: control, LO nasal spray (LS), and indoor exposure to LO (LE)] during the prepubertal period. The body weights of the animals were also compared every 3 days until the day of vaginal opening, at which time gonadotropin levels and internal organ weights were assessed.

Results

The LS group showed early vaginal opening at 33.8 ± 1.8 days compared with the control (38.4 ± 2.9 days) and LE (36.6 ± 1.5 days) groups. Additionally, luteinizing hormone levels were significantly higher in the LE and LS groups than in the control group. Body weights did not differ significantly among the groups.

Conclusions

Exposure to an exogenic simulant via inhalation during the prepubertal period triggered early puberty onset in female rats. Further evaluation of exposure to other endocrine-disrupting chemicals capable of inducing central precocious puberty through the skin, orally, and/or nasally is warranted.

Background

Central precocious puberty (CPP) describes the early activation of the hypothalamic–pituitary–gonadal (HPG) axis, which leads to the rapid progression of bone age, early menarche, reduction in final adult height, and the appearance of secondary sexual characteristics before 8 and 9 years of age in girls and boys, respectively (1). Traditionally, CPP is accompanied by intracranial lesions, including optic glioma, pilocytic astrocytoma, hydrocephalus, Rathke's cleft cyst, and pituitary adenomas, in 40–90% of boys and < 10% of girls (2, 3). The gonadotropin-releasing hormone (GnRH) stimulation test is used for diagnosing CPP, and the basal luteinizing hormone (LH) level is considered as a valuable tool to assess pubertal state (4). CPP treatment was introduced in 1980, and dosing of a recombinant GnRH agonist every 4 weeks or 3 months leads to increases in the final adult height and delayed menarche (2, 3). Improving the

final adult height of children with CPP is one of the major issues during treatment (5, 6). However, the incidence of precocious puberty is rapidly increasing, and examination and treatment of this condition are becoming a major burden because of the associated medical expenses, although the cause this condition remains unknown (2, 3).

Environmental hormones, i.e., endocrine-disrupting chemicals, were recently suggested to contribute to the onset of puberty in childhood (7, 8), and animal studies demonstrated that endocrine-disrupting chemicals accelerate pubertal onset (9, 10). Additionally, previous reports showed that lavender oil (LO) and tea tree oil are associated with prepubertal gynecomastia in boys (11, 12). Moreover, cases of premature thelarche that resolved after cessation of exposure to lavender-containing fragrance have been reported (13, 14), and an *in vitro* study showed that components of LO, including linalool and linalyl acetate, activate estrogen-related gene expression in human cell lines (13). However, studies of the absorbance of these materials in sufficient amounts and their effect on breast growth have not been performed. A previous study suggested that smell sensation can be transmitted to the central nervous system, thereby facilitating the bypass of inhaled molecules via the nasal pathway of the blood–brain barrier (15). There are several opportunities for inhalation of numerous endocrine-disrupting chemicals from estrogenic sources in cosmetics, perfumes, air fresheners, and scented candles/diffusers using aromatic oils, which can directly affect olfactory stimulation of the neuroendocrine system. In this study, we tested whether continuous inhalation of LO affects early gonadotropin activation and precocious puberty. Given the difficulty of limiting or measuring nasal exposure to endocrine-disrupting chemicals in humans, we investigated the effects of continuous inhalation of LO on pubertal onset and gonadotropin hormone levels in an animal model and compared them to control conditions.

Methods

Animals and Experimental Design

To obtain study animals, rats were bred using male and female Sprague–Dawley rats. From birth onward, we maintained an indoor temperature of 22°C (humidity: 30–70%) and controlled illumination (12-h light/dark cycle) to allow breeding in a constant environment along with free access to water and food. On day 18 after birth, we identified 15 immature females and randomly divided them into three groups: olfactory stimulation groups 1 and 2 and a control group (n = 5/group). We used 100% pure LO obtained from *Lavandula angustifolia* (NOW Foods, Bloomingdale, IL, USA) for all experiments. Group 1 was treated by indoor exposure to LO (LE) via an LO diffuser in the cage using an LO-soaked puff (changed daily) along with daily exposure to 0.9% NaCl spray. For Group 2, LO was administered as a nasal spray of aromatic LO (LS) once daily. The control group was treated with a single exposure to a nasal spray (0.9% NaCl) daily. The dose of one spray of LO or 0.9% NaCl ranged from 72–125 μL. The body weight of the animals was measured every 3 days from postnatal day 18 to the day of vaginal opening (VO). This study was approved by the Southwest Medi-Chem Institute, Institutional Animal Care and Use Committee (approval no. SEMI-20-001).

Analysis of VO

All study groups were evaluated for VO time as an indicator of pubertal initiation at a fixed time (09:00 h) daily. The day of VO was recorded, and VO timing was compared between the three study groups.

Euthanasia and Hormone Assays

After VO was observed in each rat, we measured serum luteinizing hormone (LH), follicle-stimulating hormone (FSH), and estradiol levels to compare hormone concentrations between study groups. The endpoint of the experiment was defined as VO occurring in the last rat. For this process, truncal blood was collected into ice-cold ethylenediamine tetraacetic acid-containing tubes after decapitation, after which the tubes were centrifuged, and plasma samples were collected and stored at − 20°C until analysis. The plasma levels of LH, FSH, and estradiol of each rat were measured using enzyme-linked immunosorbent assay kits (cat. no. MBS764675, MBS2502190, and MBS263850, respectively; MyBioSource, Inc., San Diego, CA, USA) according to manufacturer's instructions.

Measurement of Organ Weight

After euthanasia, we measured the weight of the ovaries, spleen, kidneys, and liver. The organ weight was then modified by body weight and presented as tissue weight per 150 g body weight.

Statistical Analysis

Data are presented as the mean ± standard deviation, and statistical analyses were performed using SPSS software (v.26.0; SPSS, Inc., Chicago, IL, USA). Statistical significance was determined by Kruskal–Wallis test and one-way analysis of variance for multiple-group comparisons and Mann–Whitney *U* test for comparisons between two groups. Statistical significance was defined at $p < 0.05$.

Results

Effect of Olfactory Exposure to LO on VO and Pubertal Onset

VO occurred earlier in the LE (33.8 ± 1.8 days; $p = 0.013$) and LS (36.6 ± 1.5 days; $p = 0.026$) groups than in the control group (38.4 ± 2.9 days) (Table 1), and VO in the LE group occurred significantly earlier than in the control group. However, there was no significant difference between the LS and LE groups with respect to VO timing ($p = 0.37$). Almost all rats in the LE group experienced VO at 33 days, the LE group showed VO at 16 days, and the control group mostly showed VO between 38 and 41 days (Fig. 1).

Table 1
Comparison of VO day among different study groups.

Group	Control	LS	LE
Rat	VO (Age, days)	VO (Age, days)	VO (Age, days)
1	41	37	37
2	41	37	33
3	38	34	33
4	38	37	33
5	34	38	33
Mean ± SD	38.4 ± 2.9	36.6 ± 1.5*	33.8 ± 1.8*
* $p < 0.001$ vs. control			
LE: exposure to diffused LO; LO: lavender oil; LS: exposure to LO as a nasal spray; SD: standard deviation; VO: vaginal opening.			

Measurement of Gonadotropin Hormone and Estradiol Levels

LH levels were significantly higher in the LE (67.6 ± 3.0 mIU/mL) and LS (64.3 ± 7.4 mIU/mL) groups than in the control group (49.9 ± 2.7 mIU/mL; $p < 0.001$ for both) (Table 2). Additionally, FSH levels were significantly higher in the LE (50.9 ± 9.1 ng/mL) and LS (51.4 ± 7.1 ng/mL) groups than in the control group (35.2 ± 3.7 ng/mL; $p = 0.009$ and $p = 0.011$, respectively). Estradiol levels were elevated in both the LE (4.9 ± 1.4 ng/mL) and LS (5.3 ± 1.4 ng/mL) groups relative to the control group (3.9 ± 0.8 ng/mL), although the differences were not significant ($p = 0.33$ and $p = 0.55$, respectively).

Table 2
Comparison of hormone levels among different study groups.

Hormones	Control	LS	LE
LH (mIU/mL)	49.90 ± 2.71	64.3 ± 7.4**	67.6 ± 3.0**
FSH (ng/mL)	35.24 ± 3.65	51.4 ± 7.1*	50.9 ± 9.1*
Estradiol (ng/mL)	3.9 ± 0.8	5.3 ± 1.4†	4.9 ± 1.4†
Data are presented as the mean ± standard deviation (n = 5).			
* p < 0.05 vs. control			
** p < 0.001 vs. control			
† not significant vs. control			
FSH: follicle-stimulating hormone; LE: exposure to diffused LO; LH: luteinizing hormone; LO: lavender oil; LS: exposure to LO as a nasal spray.			

Measurement of Body and Organ Weights.

Measurement of the body weight of rats in the control, LE, and LS groups every 3 days from postnatal day 18 until VO revealed no significant differences between the three groups (Fig. 2 and Table 3). The weights of the ovaries, kidneys, liver, and spleen after VO showed no significant differences among the three groups; however, the weight of the kidneys per 150 g body weight increased significantly after VO in the LE group (1.926 ± 0.154 g) compared with that in the control (1.664 ± 0.077 g; p < 0.01) and LS (1.694 ± 0.154 g; p < 0.05) groups (Table 4).

Table 3
Comparison of average body weights of female rats among the three groups on different PNDs.

PND	Control	LS	LE
21	43.8 ± 2.6* g	44.0 ± 3.1 g	41.6 ± 1.7 g
27	76.6 ± 4.6* g	75.6 ± 3.6 g	72.5 ± 2.8 g
33	115.4 ± 7.6* g	110.2 ± 4.9 g	110.8 ± 5.7 g
Data are presented as the mean ± standard deviation (n = 5).			
* not significant			
LE: exposure to diffused LO; LO: lavender oil; LS: exposure to LO as a nasal spray; PND, postnatal day.			

Table 4
Comparison of organ weights of female rats among the three groups.

Organ[a]	Control	LS	LE
Liver	7.658 ± 0.276[†]	7.642 ± 0.175	7.778 ± 0.346
Spleen	0.570 ± 0.046[†]	0.579 ± 0.064	0.639 ± 0.119
Kidney	1.664 ± 0.077	1.694 ± 0.154	1.926 ± 0.154[*, **]
Ovary	0.326 ± 0.054[†]	0.321 ± 0.043	0.368 ± 0.038
Data are presented as the mean ± standard deviation (n = 5).			
[a] Tissue weight (g) per body weight (150 g)			
[*] p < 0.05 vs. LS			
[**] p < 0.01 vs. control			
[†] not significant			
LE: exposure to diffused LO; LO: lavender oil; LS: exposure to LO as a nasal spray.			

Discussion

Here, we found that persistent exposure to LO is associated with HPG axis activation and early pubertal onset. We observed early VO in rats persistently exposed to LO as compared with that in the control group. Additionally, serum LH and FSH levels were significantly higher in the LE and LS groups than in the control group. To the best of our knowledge, no previous studies have reported an association between pubertal onset and persistent olfactory exposure to LO in an animal model. Nasal inhalation is an important source of iatrogenic sex-hormone exposure, and olfactory exposure to LO may result in LO delivery to the central nervous system and bloodstream to induce an iatrogenic effect of estrogen.

Several studies have shown that LO is effective at reducing menopausal symptoms and supporting healthy sleep (16, 17). Additionally, previous studies reported that LO does not increase estrogen levels in adults (18); in contrast, in the present study, we could not conclude that LO exposure did not affect estrogen activity. Although we observed no differences in estradiol levels between the LE and control groups, the LE group showed early pubertal onset and significantly increased LH and FSH levels. Furthermore, the LS group showed no significant differences in VO compared with the control group. Therefore, the amount and persistence of LO exposure may determine pubertal onset.

A previous animal study reported that percutaneous injection of LO when performing uterotrophic assays on immature rats results in significantly reduced body weight gain after 3 days compared with that in the control group and a group administered 17α-ethinyl estradiol (19). However, the authors only assessed

weight gain and organ-weight-to-terminal-body-weight to evaluate the presence of an estrogenic effect, and did not compared hormone levels or VO timing. The different administration modalities may have been responsible for the reported differences in body weight gain between the LO injection group and group undergoing oral estrogen administration. In the present study, we found no differences in organ weight, including the ovaries, and body weight gain between groups, despite the apparently different VO and gonadotropin levels. Interestingly, the kidney tissue weight was significantly increased only in the LE group, supporting an association between physiological LO-specific effects and endocrine effects. A recent study showed that LO exposure affects renal restoration in a dose-dependent manner by decreasing antioxidant signals and inflammatory cytokine levels, as well as by inhibiting apoptosis (20). In the present study, we measured neither renal function nor nephron numbers and used only renal tissue weight as an indicator of the positive effect of LO exposure. Therefore, increased renal tissue weight may indicate a renal burden related to LO excretion.

Four studies have reported a total of 11 pediatric patients (7 males and 4 females) showing premature thelarche in females (age range: 14 months to 7 years, 9 months) and gynecomastia in males (age range: 4 years, 5 months to 10 years, 1 month) after using an LO-containing product (11–14). Ramsey et al. (13) reported that patients showed symptomatic improvement after discontinuation of LO exposure accompanied by no abnormal laboratory findings. These reports suggest the estrogenic effect of topical preparation of LO. Additionally, *in vitro* studies showed that LO (or the LO components linalool and linalyl acetate) exerts an estrogenic effect by stimulating transcription of the estrogen receptor *α*- (11, 13). The peripheral hormones or signals transmitting to GnRH neurons may lead to GnRH secretion and stimulation of pituitary gonadotropins and gonadal sex steroids (8). Given our observation of early activation of the HPG axis after olfactory exposure to LO, further investigation of the effect of LO on gene activation related to GnRH synthesis or secretion may explain the associations between early activation of the HPG axis and LO exposure.

We focused on the effects of LO exposure through olfactory stimulation and not via oral ingestion or topical application. One limitation of this study is its small sample size; thus, further studies are required to validate the findings. We observed significant elevation of gonadotropin levels not only in the LE group but also in the LS group, suggesting that repetitive olfactory exposure affected the manifestation of LO-specific physiological effects. Several studies reported that essential LO affects anxiety when administered via the oral or nasal routes (21–23). However, LO exposure via oral ingestion in food is not as common as skin absorption of various LO-containing cosmetic products or LO inhalation. Nevertheless, inhalation of environmental LO is difficult to quantify in humans, and epidemiological surveillance data for the sole effect of LO inhalation in children undergoing precocious puberty are unavailable. Furthermore, the frequency and duration of exposure to LO inhalation are highly variable, and the effect of LO following skin exposure on children remains inconclusive because of the variable amounts of LO to which the skin is exposed, and difficulties associated with follow-up to assess long-term effects (24).

Conclusions

This study showed the effect of olfactory stimulation by LO on the early onset of puberty. These results suggest that avoidance of LO exposure to minimize unnecessary iatrogenic estrogen effects from fragrances, diffusers, and perfumes can prevent early stimulation of the HPG axis, particularly in younger children. Further *in vitro* evaluation of LO-related effects on central kisspeptin signaling may reveal the mechanisms associated with early activation of the HPG axis by persistent olfactory exposure to LO.

Abbreviations

C: Control, LS: Spray of lavender oil, LE: Exposure to lavender oil, LH: Luteinizing hormone, FSH: follicle-stimulating hormone, VO: vaginal opening, LO: lavender oil, EDCs: endocrine-disrupting chemicals, CPP: central precocious puberty, HPG: hypothalamic–pituitary–gonadal

Declarations

Ethics approval and consent to participate

This animal study was approved by Southwest Medi-Chem Institute, Institutional Animal Care and Use Committee (approval number: SEMI-20-001).

Consent for publication

Not applicable.

Availability of data and materials

All data generated or analysed during this study are included in this published article.

Competing interests

The authors declare no conflict of interest.

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Author contributions

Substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data: Yoo-Mi Kim. Drafting the article: Yoo-Mi Kim & Han Hyuk Lim. Both authors critically revised the manuscript and approved the final version for publication.

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Figures

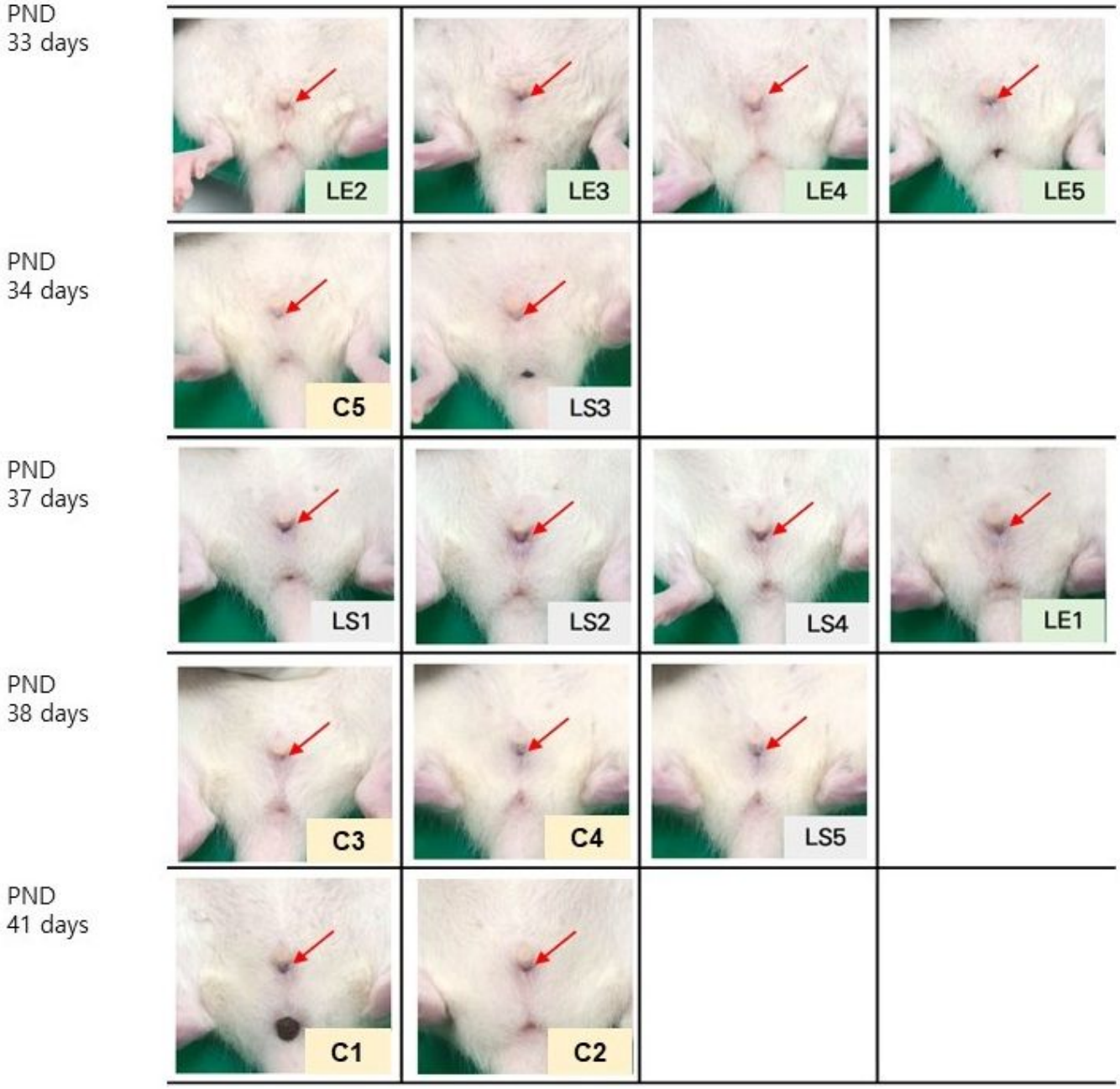

Figure 1

Different vaginal opening onset times according to olfactory exposure to lavender oil. C: control; LE, exposure to diffused LO; LO, lavender oil; LS: exposure to LO via nasal spray; VO, vaginal opening.

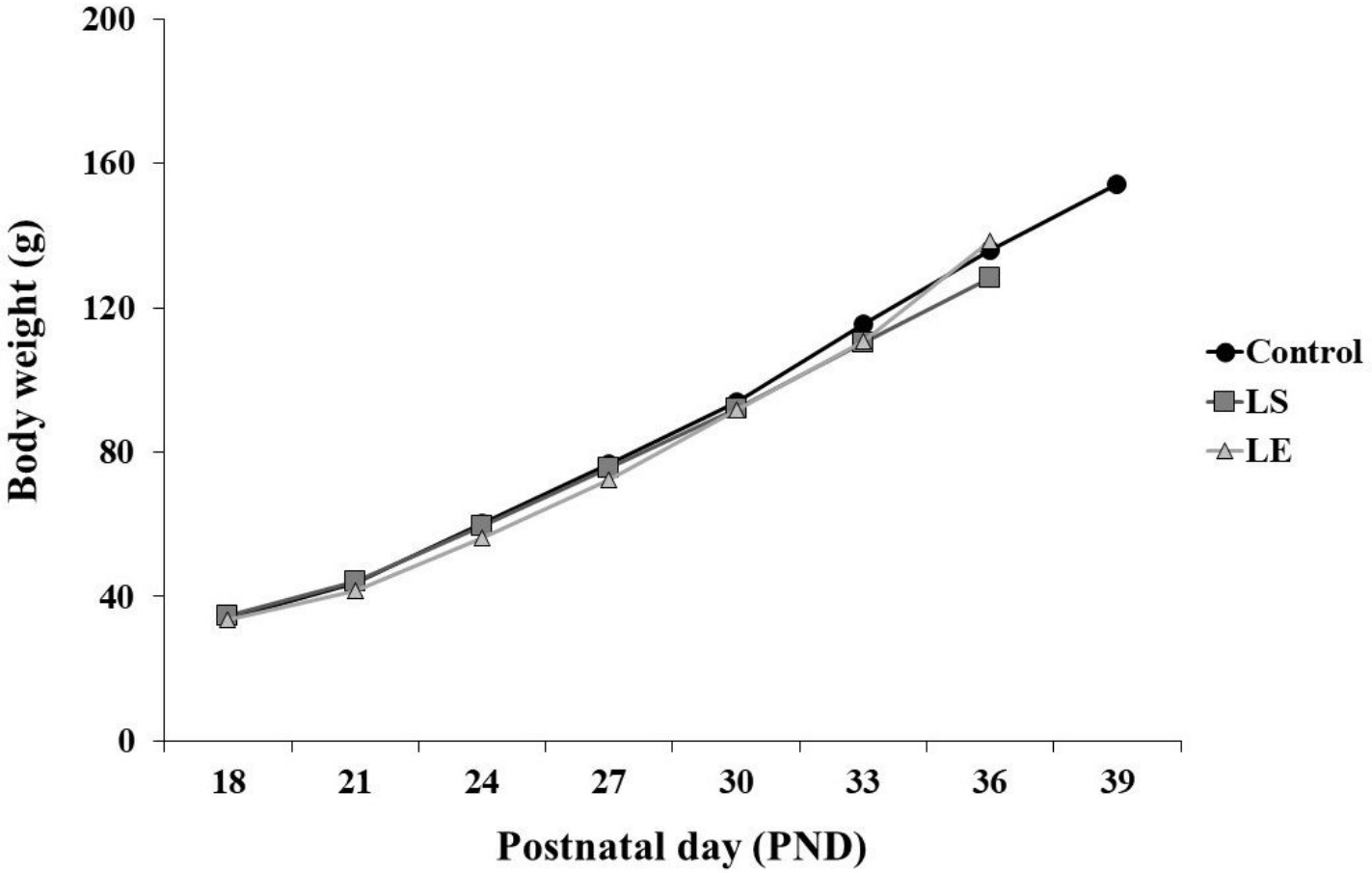

Figure 2

Changes in mean body weight from postnatal day 18 and after lavender oil exposure. LE, exposure to diffused LO; LO, lavender oil; LS: exposure to LO via nasal spray; PND, postnatal day.