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ORIGINAL RESEARCH

Ethinylestradiol Transfer into Breast Milk of Women Using Low-Dose Combined Hormonal Contraception Is Negligible

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Introduction: Few studies have examined the transfer of Ethinylestradiol (EE) into breastmilk in women using Combined Hormonal Contraception (CHC). Most studies are decades old, from when EE doses (≈50 µg) were higher than today (≤35 µg).

Methods: Here, we asses EE levels in milk from breastfeeding women on CHC with low-dose EE (15–35 µg/day EE) using mass spectrometry (MS). Our study included 14 breastfeeding women: 6 using oral CHC (15, 20, or 30 µg/day EE), 7 using a vaginal ring (15 µg/day EE), and 1 using a transdermal patch (35 µg/day EE). A control group of 8 breastfeeding women not using hormonal contraceptives was also included. All participants completed a background questionnaire and provided 5mL of breast-milk. Samples were lyophilized and extracted with methyl tert-butyl ether (MTBE) for Liquid Chromatography-Mass Spectrometry (LC-MS) analysis. EE levels were assessed using high-resolution LC-MS, with a limit of quantification (LOQ) of 3.5 ng/mL.

Results: No measurable peak of the compound was found in any of the CHC users. To confirm that EE was not lost during sample preparation, pure EE was added to control breast milk and successfully detected.

Conclusion: These findings suggest that EE transfer into breastmilk is less than 3.5 ng/mL, and therefore negligible compared to endogenous estradiol. This first LC-MS–based study provides novel evidence supporting the lactation safety of modern low-dose CHC, though larger studies with lower detection limits are needed for confirmation.

Plain Language Summary: Our findings suggest that the breast milk transfer of Ethinylestradiol (EE) from current low-dose combined hormonal contraception (CHC) is negligible. As such, our findings suggest that modern low-dose CHC can be safely used during breastfeeding, offering reassurance to both mothers, and healthcare providers.

Keywords: combined hormonal contraception, ethinylestradiol, breast-milk, lactation safety, mass spectrometry

Introduction

After delivery and during breastfeeding, the recommended oral hormonal contraceptives are progesterone-only pills (POP).[1] However, a common side-effect of POP is withdrawal bleeding (WB), breakthrough-bleeding (BTB), and spotting, occurring in about 40% of users, compared to only 10% with Combined Hormonal Contraception (CHC).[2] This bleeding, negatively affects many women, causing 25% to discontinue POP use.[3] WB and BTB are not a medical concern, but they do justify CHC use during breastfeeding, if there is no risk of venous thromboembolism (VTE).[4,5]

A review comparing CHC vs POP use during breastfeeding found only two studies, which have lead to inconsistent guidance.[6] On the one hand, the American College of Obstetricians and Gynecologists (ACOG) allows CHC use from six weeks postpartum.[7] The ACOG relies on a study comparing 64 women using CHC (35 µg EE) and 63 women using POP,

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which found no difference in milk volume between the groups[8] In contrast, the World Health Organization (WHO) recommends CHC use only six months postpartum.[9] The WHO guidance is based on research showing that CHC with 30 mg EE reduce milk volume, although it does not affect infant growth.[10] Paradoxically, from 6 weeks to 6 months postpartum, about half of physicians prescribe CHC, while the other half do not, due to concerns that Ethinylestradiol (EE) may pass into breast milk and harm the infant.[11] The main concern associated with CHC during breastfeeding, as perceived by physicians, is a potential decrease in breast-milk production, gynecomastia in the infant, and a combination thereof with thrombosis in the child.

Few studies have examined the transfer of EE into breast-milk. A 1978 study by Nilsson et al assessed the transfer of EE into breast-milk.[12] Breastfeeding women were given EE pills at two doses: 50 µg and 500 µg. EE was not detected in the milk of those using the 50 µg dose—likely because levels were below the detection limit of the method used. In contrast, EE was found in the milk of women taking the 500 µg dose, with milk concentrations reaching 100 ng (or 0.02% of the maternal dose). Notably, this remains the only study to directly measure EE levels in breast milk. Another study examined women taking 50–200 µg of EE, and found no estrogen in the infants nor any effect on growth.[13] The researchers concluded that, even if EE transfers into breast milk, it does so only in trace amounts and has no measurable impact on the baby. Other studies have shown that maternal estrogen intake does not affect infants. For example, one study followed 48 infants for eight years whose mothers used combined contraceptives and found no differences in height, weight, health, or cognitive development compared to other children.[14] Recently, a review article noted that most of these studies were conducted over 50 years ago when EE doses were 50 µg.[15] In contrast, modern CHC pills contain much lower doses of EE (under 30 µg). The review recommended that, due to the limitations of earlier studies and the need for effective postpartum contraception, future research should focus on breastfeeding women using current low-dose combined hormonal methods—including pills, patches, vaginal rings, injections, and others.

While early studies provided valuable insights, they were based on higher doses of ethinylestradiol (EE) that were standard at the time. These dosages (50 µg) are no longer applicable, as modern combined hormonal contraceptives contain significantly lower doses of EE (under 30 µg). This change in dosage necessitates a re-examination of EE transfer into breast milk. The existing uncertainty is also reflected in the varying guidance from health organizations: while the American College of Obstetricians and Gynecologists (ACOG) permits the use of CHCs as early as six weeks postpartum, the World Health Organization (WHO) recommends waiting until six months postpartum, underscoring the urgent need for current and comprehensive data.

In this study, we assess EE levels in the breast milk of women using CHC administered orally, vaginally, or transdermally. As a control, we also analyzed breast milk from women not using hormonal contraception. Our objective is to determine if EE does indeed transfer into breast-milk, at any level measurable using mass spectrometry.

Methods

Breast-Milk Samples

This study analyzed the self-expressed milk from breastfeeding women using various CHC formulations with EE. Breast-milk from women not using hormonal contraception served as a control. All samples were frozen immediately after self-expression, and kept at −18°C until further preparation.

This study was non-interventional, and no contraception was prescribed by the authors. Breastfeeding women were recruited via social media and enrolled after completing a background questionnaire (Supplementary Figure 1). The questionnaire confirmed that all women were at least six weeks postpartum and were willing to provide a 5 mL breast-milk sample. The questionnaire also confirmed that CHC users, self-expressed milk within 2–10 hours of the last oral dose, five days after NuvaRing insertion, or 5 days after transdermal patch application. The timeframe for milk collection was based on the long half-life ($T_{1/2}$) of EE ranging from 13 to 17 hours. In addition, previous studies showed that EE levels remained high for multiple hours after intake.[16] Peak levels (~100%) were expected in the vaginal-ring and transdermal-patch samples, and high-levels (>60%) were expected in the oral-pill samples. Finally, the questionnaire collected additional information including age, height, weight, and use of other medication.

This study was approved by the Helsinki Committee of Ziv Medical Center, Safed (Permit number 0123–22-ZIV). In accordance with the Declaration of Helsinki, all participants provided informed consent.

Sample Preparation

Extraction and analysis of EE were performed using a slightly modified Matyash method.[17] Briefly, 5 mL of breast-milk was lyophilized at −110°C, yielding, on average, 800 mg of lyophilized dry milk-powder. For each sample, 300 mg of powder were transferred to separate 2 mL Eppendorf tube for extraction. EE and its potential metabolites were extracted using 1 mL of pre-cooled (−20°C) methanol:methyl-tert-butyl-ether (MTBE) solution (1:3, v/v) containing the following internal standards: 0.1 µg/mL of phosphatidylcholine (17:0/17:0) (Avanti Polar Lipids, Alabaster, AL, USA), 0.4 µg/mL of phosphatidylethanolamine (17:0/17:0) (Avanti Polar Lipids, Alabaster, AL, USA), and 2 µg/mL cannabidiol (synthesized and recrystallized in our lab, 100% purity). Tubes were vortexed, then sonicated for 30 minutes in an ice-cold sonication bath, with brief vortexing every 10 minutes. After sonication, 0.5 mL of UPLC-grade water:methanol solution (3:1, v/v) were added. Then, tubes were centrifuged at 17,950 x g, and the upper organic phase was collected. The remaining polar phase was re-extracted as described above with an additional 0.5 mL of MTBE. Both organic phases were combined, dried using SpeedVac (Savant, Thermo Scientific, USA), and dried sample extracts were stored at −80°C until further analysis. Importantly, the study was blinded, and all samples were labeled so as not to infer potential bias.

Sample Stability

To confirm EE stability, and to ensure that it is not destroyed during sample preparation, control breast-milk (ie, from a woman not using CHC) was spiked with EE to a final concentration of 100, 50, 25, 12.5, 6.25, 3.125, 1.56, and 0.78, 0.39, 0.195 and 0.0975 µg/mL EE (Databiotech). These samples, also used to evaluate the extraction consistency, underwent the same extraction process, as detailed above.

Mass-Spectrometry (MS) Coupled with Liquid Chromatography (LC)

High-resolution mass spectra were obtained using a Xevo G2-XS QTof mass spectrometer (Waters), equipped with an ACQUITY UPLC system (Waters). Data were acquired using resolution mode under positive electrospray ionization (ESI). Acquisition range was set to 50–1500 m/z, with a capillary voltage of 1.5 kV, and a cone voltage of 40 V. Each scan time was 1.0 seconds. All data were acquired in centroid mode by the MassLynx NT4.1 software and analyzed by QuanLynx program (Waters).

For analysis, dried sample extracts (from 3 mL milk) were re-suspended in 100 µL mobile phase B (see composition below), centrifuged again at 17,950 x g at 4°C for 10 min, filtered through 0.2 µm-pore filters, and transferred to HPLC injection vials.

Samples were injected into a BEH C18 column (1.7 micron particle size, 2.1 × 50mm, Waters) which was maintained at 30°C throughout the analysis. Unless specified otherwise, all solvents were obtained from Merck (Rehovot, Israel).

The mobile phase solvents consisted of: (A) water with 0.05% formic acid, (B) Acetonitrile with 0.05% formic acid, and (C) Methanol. Flow rate was constant at 0.3 mL/min, with constant solvent C elute at 3% for the whole run. The gradient elution began with 1% solvent B until 1.5 minutes, then increased to 48% at 2.25 minutes, and reached 96% at 2.7 minutes. This concentration remained stable for another 3.9 minutes, until 7.05 minutes. Then, solvent B was reduced back to 1% at 10 minutes, and the column was re-equilibrated for another 3 minutes. The gradient elution profile is shown in Supplementary Table 1.

MS Calibration Standard

The control mass of EE (Databiotech) was identified using the following MS parameters: the source and de-solvation temperatures were maintained at 150°C and 400°C, respectively. The capillary voltage was 1.5 kV, and cone voltage was 40 V. Nitrogen was used both as desolvation gas (800 L/h), and cone gas (150 L/h). Detection was performed in positive-ion mode using ESI technique, and quantification was performed with MS1 centroid mode.

To assess the precision and accuracy of our methodology, calibration standards and quality control (QC) samples was prepared. A stock solution of EE (Databiotech) was prepared at 1 mg/mL in acetonitrile, followed by serial dilution with

acetonitrile immediately before use, to concentrations of 100, 50, 25, 12.5, 6.25, 3.125, 1.56, and 0.78, 0.39, 0.195 and 0.0975 µg/mL. Calibration standards were used to generate a calibration curve, which was calculated using a weighted linear regression model with weighted linear curve fit equation, 1/x. Calibration curves were considered valid if the correlation coefficient (r) exceeded 0.98.

Results

Breast-Milk Samples

This study analyzed the self-expressed breast-milk of women (N=14) using various formulations of CHC with EE. As a control, the study used the self-expressed breast-milk of women (N=8) using no hormonal contraception. The composition of each CHC, as well as the EE dose, and number of breastfeeding women, is detailed in Table 1.

Each of the women completed a background questionnaire, before providing a milk sample. Table 2 lists the self-reported age of the breastfeeding women, the age of her infant, as well as her body-mass index (BMI). No significant differences were observed among the CHC user and control group. The CHC oral pill users reported that breast-milk was pumped 2–10 hours after the last pill. The CHC Nuvaring users reported that at least 5 days have passed since ring insertion. The CHC transdermal patch users reported that at least 5 days have passed since patch application. The self-reported medications included: vitamin D, vitamin B12, Prenatal Multi-vitamin, Bee pollen, Moxyvit Forte (Amoxicillin), Relvar Ellipta (Fluticasone/Vilanterol), Bilaxten (Bilastine), Montelukast Trima (Montelukast), Euthyrox (Levothyroxine), and Viepax (Venlafaxine). The self-reported medication were not suspected of altering EE levels, through pharmacokinetic interactions, as posited by the Drug Interaction Checker Medscape (http://reference.medscape.com/drug-interactionchecker, accessed on June 2025).

Table 1 Combined Hormonal Contraception (CHC) Used

CHC	Progestin		Estrogen	Formulation	Users (N)
Brand Name	**Generic Name**	**Dose (mg)**	**Ethinylestradiol Dose (µg)**		
Feminet	Desogestrel	0.15	20	Oral	1
Yasmin	Drospirenone	3	30	Oral	1
Yaz	Drospirenone	3	20	Oral	1
Microgynon	Levonorgestrel	0.15	30	Oral	2
Minesse	Gestodene	0.06	15	Oral	1
Novaring	Etonogestrel	0.120	15	Vaginal	7
Evra Patch	Norelgestromin	0.150 (daily)	35 (daily)	Transdermal	1

Table 2 Women Characteristics

	CHC Users*	Non-Users#	P-value^	Range
N	**14**	**8**		
Woman age (years)	27.2 (±3.86)	30.6 (±7.22)	0.251	22–44
Infant age (months)	8.1 (± 4.18)	7.1 (± 3.87)	0.504	1.5–19
Body mass index (kg/m^2)	25.2 (± 4.07)	22.3 (±2.89)	0.075	18.9–35.2

Notes: *Women that used Combined Hormonal Contraception (CHC). #Women that did not use hormonal contraception. ^P-values below 0.05 were considered statistically significant.

Each of the women provided a 5 mL breast-milk sample. Importantly, the milk samples were frozen immediately after self-expression, and kept at −18°C until further use. All samples were prepared for MS analysis as described in the methods. Lyophilization of 5 mL of breastmilk, resulted in ~800 mg of dry milk-powder per tube. Extraction using the modified Matyash method[17] with 800 mg dry milk-powder, yielded less than 1 mg of solids for analysis using MS.

Mass Spectrum (MS) Calibration Standard

Figure 1 shows the spectrum of EE standard. A prominent peak of EE was identified at 279.1748 gr/mol ($[M-H_2O+H]^+$), and was observed in full certainty in the standard. The spectrum also showed a minor peak at the expected molecular mass of EE at 297.1848 gr/mol ($[M+H]^+$) close to the theoretical monoisotopic mass 297.1776 gr/mol). The prominent peak was due to fragmentation during ionization, and loss of a water molecule (297.17–279.17 = 18 gr/mol). The mass spectrum of EE suffered from fragmentations, and measurement was challenging, as reported earlier.[18] Notably, EE derivatization with Dansyl Chloride failed to improve sensitivity significantly (unpublished data, Soliman Khatib lab), despite earlier successful reports.[19] In addition, negative electrospray ionization (ESI) also failed to improve sensitivity significantly (unpublished data, Soliman Khatib), despite earlier successful reports.[20]

Figure 2 shows the calibration curve of the EE standard. Figure 2 shows a clear correlation between the amount of EE injected, and the area under the curve (AUC) of the EE peak at 279.1744 gr/mol that appeared in the spectrometer. The calibration was performed with serial dilutions of the EE standard, and the R-squared value was 0.9942, indicating an excellent fit. The lowest measurable concentration of EE was 0.0975 µg/mL (ie, ~100 ng/mL). Any further dilution did not result in a peak.

LC-MS of Samples

Remarkably, none of the extracted breast-milk samples showed any peaks for EE. Figure 3 shows examples of LC-MS spectra of milk-samples derived from one of the 14 women using CHC (Figure 3A), and from one of the 8 control women, not using any CHC (Figure 3B). Figure 3 also shows LC-MS spectra of one of the control women spiked with EE (100, 50, 25, 12.5, 6.25, 3.125, 1.56, and 0.78, 0.39, 0.195 and 0.0975 µg/mL), and the standard pure EE (100 ng/mL EE). Only the control sample spiked with EE standard (Figure 3C), and the EE standard sample (Figure 3D) display a peak at 4.02 minutes, and corresponding to EE with 279.1744 gr/mol. No other milk-samples derived from any of the women displayed EE peak corresponding to EE.

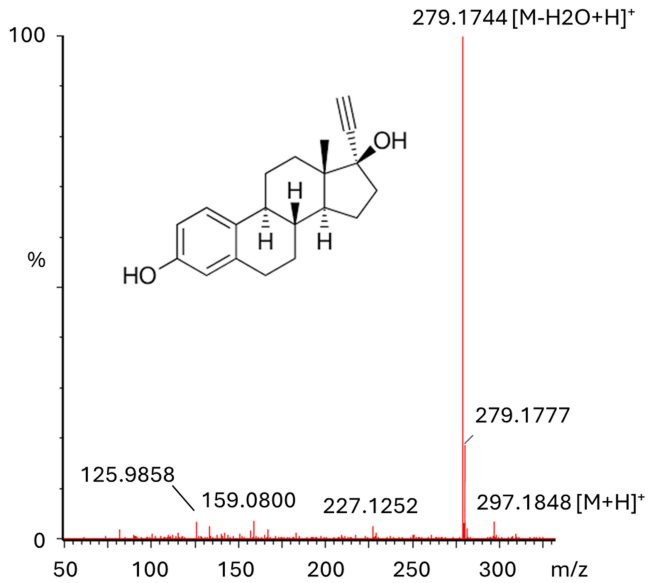

Figure 1 Mass spectrum of Ethinylestradiol (EE) standard. Shown is the spectrum of pure EE. Only a minor peak at 297.1848 gr/mol corresponds to the expected mass, and the major peak at 279.1744 gr/mol corresponds to the dehydrated form. Notably, fragmentation is common.

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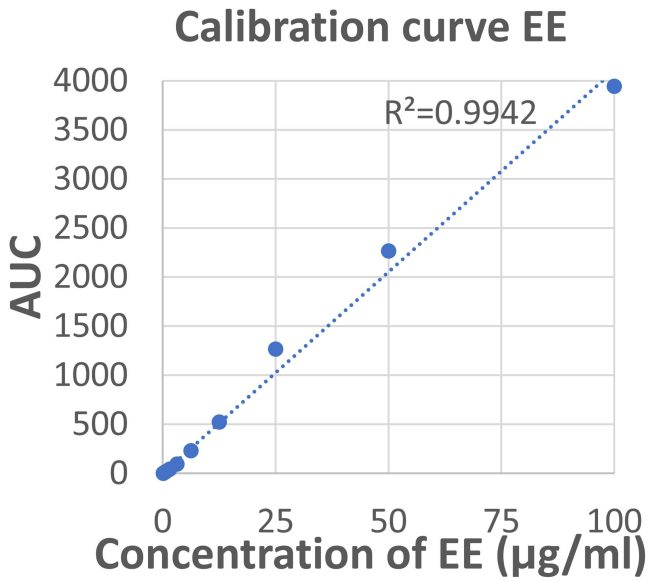

Figure 2 Calibration curve for Ethinylestradiol (EE) standard. Shown is the AUC of increasing EE concentrations (100, 50, 25, 12.5, 6.25, 3.125, 1.56, 0.78, 0.39, 0.195, and 0.0975 µg/mL). Note the high correlation between AUC and EE concentration inserted in the MS.

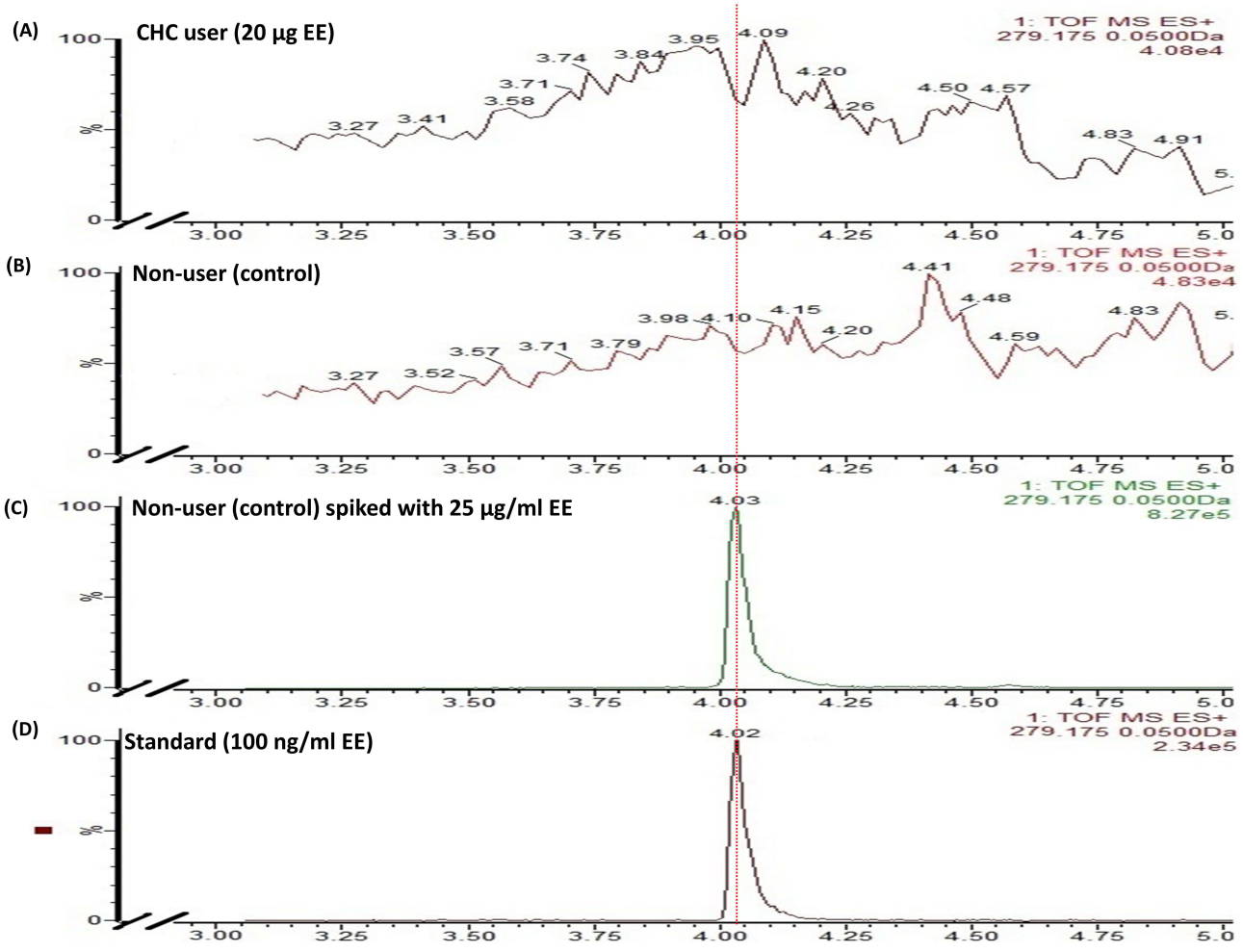

Figure 3 LC-MS of extracted milk samples. Shown are samples extracted from breast-milk that were subjected to LC-MS of (**A**) a woman that used CHC, (**B**) a control woman that used no CHC, (**C**) another woman that used no CHC, laced with EE. Shown in (**D**) is the LC-MS spectrum of a standard EE sample. The x-axis is the elution time until peak appearance (in minutes) and corresponds to a peak of EE at 279.17 gr/mol.

Notably, the presence of a compound with the molecular weight of 279.0948 gr/mol in the samples did not correspond to EE, as was found in milk samples of CHC-users as well as non-users. This substance did not correspond to EE as attested by its presence in the breastmilk of those who did not use CHC.

The limit of detection (LOD) of EE with LC-MS in spiked milk was 0.0975 µg/mL (~100 ng/mL). Because we concentrated milk samples from 3 mL to 100 µL (times 30), the limit of quantification (LOQ) of EE was calculated as ~3.5 ng/mL (~100/30 ng/mL). As such, our data suggest that EE did not transfer into, at a concentration higher than ~3.5 ng/mL, for any of the breastmilk samples. However, to ascertain that there is no EE in the breastmilk, studies with a larger number of participants, and a lower detection threshold, are needed.

Stability Assay

To confirm the stability and recovery of EE during sample preparation, surplus control breast milk (from a woman not using CHC) was spiked to various final concentration of EE (100, 50, 25, 12.5, 6.25, 3.125, 1.56, and 0.78, 0.39, 0.195 and 0.0975 µg/mL). These samples underwent the same extraction process, and EE was successfully detected at 279.17 gr/mol (Figure 3C). Notably, the peak area was correlated with the EE concentration (R=0.99). This confirmed that the EE peaks at 279.17 gr/mol remain intact throughout sample preparation and MS analysis, and that LOD concentrations can be detected.

Discussion

To evaluate the lactation-safety of combined hormonal contraception (CHC) during nursing, we measured the presence of Ethinylestradiol (EE) in the breast-milk of women using low-dose CHC. Using liquid chromatography – mass spectrometry (LC-MS), we found no EE concentration, exceeding 3.5 ng/mL, in milk samples CHC-users. As a control, we analyzed the breast-milk of women who nursed and did not use any hormonal contraceptives. EE with a molecular mass of 279.1711 gr/mol was only detected in samples into which we intentionally introduced EE at known quantities. In addition, we found no difference in the results of either CHC group, whether using the vaginal ring (Etonogestrel 0.12 mg/day and EE 15 µg/day), contraceptive patches (Norelgestromin 0.15 mg/day and EE 35 µg/day), or oral contraceptives (Table 1). Thus, no EE was detectable, at a concentration higher than 3.5 ng/mL, in any of the breast-milk samples. In light of our stability assay, we conclude that EE is not to be found in milk samples at a concentration above this limit.

To the best of our knowledge, our study is the first to use LC-MS to detect EE in human breastmilk samples. Our findings are less sensitive, but consistent with earlier studies that used radiolabeled EE to estimate breast-milk concentration by radioimmunoassay.[12] That study also found EE levels in milk of women taking CHC (50 µg EE) to be below the detection limit of 50 pg/mL. Based on the data from women using high-dose EE (500 µg), the estimated theoretical EE concentration for those using CHC (50 µg EE) was 5–20 pg/mL. The maximum theoretical dose ingested by a fully nursed infant was estimated at 10 ng – only 0.02% of the maternal dose (50 µg EE). In our study, the estimated dose ingested by infants from mothers using 15, 20, or 30 µg EE CHC is less than 3, 4, and 6 ng/day, respectively – quantities considered negligible compared to endogenous levels of estradiol (E2) in mature breast milk – that is the weighted concentrations of free E2 at 15 days postpartum – ranging from 0 to 18.5 ng/mL.[21]

Our findings are also consistent with FDA data related to Ortho Evra (EE 35 µg/day), and after application of a single transdermal patch, the steady state blood concentration (Css) of EE is estimated to 80 pg/mL (www.accessdata.fda.gov/drugsatfda_docs/label/2008/021180s026lbl.pdf, accessed in July 2025). In comparison, oral CHC (EE 35 µg/day) results in a Css of 50 pg/mL, and breast-milk levels of EE are not expected to exceed these systemic levels. A potential limitation of this study is the unexpected low sensitivity of the MS assay (<3.5 ng/mL) which restricts exact quantification. As such, our study can serve as supporting evidence to earlier findings, through a different measurement technique.

These findings suggest that EE exposure through breast-milk is minimal, supporting CHC lactation-safety. CHC is a contraception alternative with fewer bleeding issues than progestin-only pills (POP).[1] As POP discontinuation due to inconvenience is common, and can lead to unplanned pregnancies, access to alternative contraception like CHC is important. Although health organizations such as the ACOG allow the use of CHC starting 6 weeks after birth in lactating women, about 50% of physicians still avoid prescribing it due to concerns about reduced milk-supply and potential EE transfer to infants.[11] As

the potential concern of a possible decrease in the milk amount has been challenged by various studies,[6] it is crucial to ascertain that EE doe not pass into breast-milk. Our study verifies the lactation-safety of CHC, nevertheless, informed discussion with patients about the potential risks and benefits of EE are paramount in clinical settings.

Limitations

Several limitations should be noted in our study. First, we did not detect EE directly in any of the non-spiked milk samples, and MS may not be suitable for this purpose. Second, we also did not assess whether EE is metabolized into other forms of estradiol. Third, breast-milk was not collected at standardized time points following CHC intake. The average time between pill ingestion and milk collection was 4.5 hours (range: 2–10 hours), and higher EE levels may have occurred at other intervals. Fourth, the detection limit of our MS method for EE in unconcentrated milk was 100 ng/mL, but it did not exclude lower concentrations. By concentrating our milk-samples 30 times, the effective detection threshold was lowered, and the limit of quantification (LOQ) was 3.5 ng/mL. If a 6-week infant consumes 600 mL per day, this corresponds to 2.1 μg EE/day. If an 6-months infant consumes one liter of breast-milk per day, this corresponds to 3.5 μg EE/day, and more studies using more sensitive detection methods, and other internal-standards, are warranted to fully assess EE transfer. Finally, as our study included a relatively small number of participants, statistical power is limited, which may affect the precision of our conclusions and interpretation of the findings.

Conclusion

Our study demonstrates that Ethinylestradiol (EE) from combined hormonal contraception (CHC) is not detectable in breast milk at concentrations exceeding 3.5 ng/mL. While these findings suggest minimal to no transfer of EE during breastfeeding, further studies are required using larger study populations and more sensitive analytical methods to fully confirm the safety of CHC use during lactation.

Abbreviations

BTB, Breakthrough Bleeding; CHC, Combined Hormonal Contraception; E2, estradiol; EE, Ethinylestradiol; EI, Electrospray Ionization; LOD, Limit of Detection; LOQ, Limit of Quantification; MS, Mass Spectrometry; MTBE, Methyl tert-butyl ether; LC, Liquid Chromatography; POP, Progesterone-only pills; UPLC, Ultra-performance liquid chromatography; VTE, Venous Thromboembolism; WB, Withdrawal Bleeding.

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Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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Disclosure

The authors declare no competing interests.

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