Urinary excretion of estrogenic chemicals following consumption of capsule coffee and French press coffee: A crossover study

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ABSTRACT

Background: Coffee brewed from capsules contain estrogenic chemicals (ECs) that may harm the reproductive system. However, there are no studies investigating whether consuming capsule coffee causes these ECs to present in urine.

Objective: Compare the effects of consuming capsule coffee vs. a plastic-free (French press) method on the appearance of ECs in urine.

Methods: Participants (n = 30) were randomized to consume 540 mL of capsule or French press coffee once, then switched and consumed the other coffee after washout. Urine samples were collected prior to consumption, at 6 h and 24 h. Coffee and urine samples were analyzed for nine ECs using ultra-performance liquid chromatography with tandem mass spectrometry: bisphenol A (BPA), bisphenol F (BPF), bisphenol S, di(2-ethylhexyl) phthalate (DEHP), benzophenone, 4-nonylphenol (4-NP), dibutyl phthalate, caprolactam and dimethyl terephthalate.

Results: In coffee samples, BPF (French press: 13.9 ng/mL, capsule: 16.1 ng/mL) and DEHP (capsule: 1.12 ng/mL) were present. In 6 h urine samples, the detection frequency for DEHP was 6.7% in capsule and 13.3% in French press coffee. BPF was detected in only one urine sample post-consumption.

Conclusion: Consuming capsule coffee did not increase urinary EC exposure compared to consuming French press coffee.

1. Introduction

Coffee is a popular beverage globally, particularly among US adults. Nearly 45% of 20–39 year-olds, 66% of 40–59 year-olds, and 73% of those 60-years old or older report consuming coffee [1]. The most common brewing method is via drip (50%), followed by single-serve capsule (28%) [2]. These single-serve capsule brewers (capsule coffee) feature small capsules pre-packed with ground coffee and are rising in popularity due to their convenience and flavor variety.

The use of these capsules has been the target of recent scrutiny due to the potential for harmful estrogenic chemicals (ECs) to leach into coffee, therefore increasing EC exposure [3–5]. ECs are endocrine disruptors that mimic the hormonal action of 17β-estradiol [6] and impose deleterious effects on male and female reproductive systems such as reduced spermatogenesis, endometriosis, infertility or abnormal pregnancy, prostate cancer, and developmental toxicity [7–9]. Given the ubiquity of ECs in the food supply (typically associated with plastic-based materials such as containers, wrappers, utensils, and silverware) as well as in the environment and their known health effects, efforts have been made to reduce the availability of certain ECs. For example, bisphenol A (BPA) is commonly removed from plastic water bottles, although substitutes such as bisphenol F (BPF) and bisphenol S (BPS) are still routinely used.

Coffee capsules are commonly produced using plastic material and additives such as polypropylene [10], which may cause ECs such as BPA and phthalates to migrate into coffee grounds [11,12]. This migration can be further promoted when heated under pressure [11]. Recent studies reported that coffee prepared from capsules contains a higher concentration and frequency of ECs compared to alternate (capsule-free) brewing methods [4,5,13]. A limited number of intervention studies have also demonstrated that the consumption of food or beverages
contaminated with ECs increases urinary excretion of these ECs, thereby increasing biological risk [14–16]. However, to our knowledge no study has confirmed whether consuming capsule coffee increases urinary EC exposure. The objective of this study was to compare the effects of consuming capsule coffee vs. a plastic-free brewing method (stainless-steel French press coffee) on the appearance of ECs in urine. Considering recent evidence supporting an elevated risk of EC contamination in capsule coffee [6,13], the hypothesis was that compared to consuming French press coffee, consuming capsule coffee would result in greater exposure to ECs as determined by urinary EC concentrations.

2. Methods

2.1. Study design

A 2-week randomized crossover trial was conducted with 30 healthy participants to compare urinary exposure to ECs via consumption of capsule and French press coffee. A crossover design was used because it allows each subject to serve as his/her own control and therefore limiting the influence of confounders that may impact EC exposure or metabolism such as age, genetics, behavior, and diet that would otherwise be variable between subjects. Participants were initially randomized into one of two groups: capsule or French press (n = 15 each). After a 7-day run-in period, participants consumed their assigned coffee. Following a 6-day washout, participants switched coffee types (Fig. 1). Three urine samples were collected over 24 h beginning immediately prior to coffee consumption. The study took place between September-November 2020.

2.2. Participants

Participants were recruited at the University of Connecticut in Storrs, Connecticut. Advertisements were sent to students via university email and posted on campus in various buildings to recruit both students and university employees. Inclusion criteria consisted of age 19–55 years, consuming coffee either regularly or irregularly, and willing to stop drinking coffee for the study duration (except for intervention coffee), and stop use of sunscreen, paper cups/dishes, plastic food/containers, and canned food/beverages to minimize exposure to ECs, and provide urine samples. Exclusion criteria consisted of heart, liver, kidney or endocrine disease or cancer, or taking estrogen-based birth control or estrogen therapy within the past 3 months to limit potential confounding of EC metabolism. Additionally, participants were excluded if currently smoking, reported having an adverse reaction to drinking or ceasing to drink 540 mL of coffee, allergic reaction to any ingredients to the food provided, or currently working a job requiring contact with thermal paper receipt, requiring food packaging, or plastic manufacturing to minimize EC contamination. The study protocol was approved by the University of Connecticut Institutional Review Board (H18–017) and was carried out in accordance with the Declaration of Helsinki.

2.3. Procedures

Throughout the entire study period, participants were instructed to avoid consuming any type of coffee (except for the coffee provided). On the day of the intervention, participants were asked to fast overnight (approximately 8 h) and arrived at the study center at 7:00 a.m., signed the consent form and completed a single questionnaire on sociodemographic characteristics, medical history, and coffee consumption behavior. A standardized breakfast was then offered (Table 1). Breakfast was optional and participants were not required to complete nor consume any item. After breakfast, a baseline urine sample was collected. Then, participants consumed 540 mL of their assigned coffee (with optional sugar and lactose-free creamer). Once the coffee was completed, participants were free to leave the study center and were provided lunch to-go. As with breakfast, lunch items were optional but participants were instructed to refrain from eating or drinking anything outside of the offered items for six hours after drinking the coffee. Participants collected their urine in amber glass bottles during these six hours. Eighteen hours later (from 6 h until 24 h after coffee consumption), participants were free to consume any food/beverage that adhered to the protocol (see avoided items listed in the inclusion criteria), and were instructed to collect their urine in a separate amber glass bottle. Twenty-four hours later, participants returned their urine samples (6- and 24-hour) to the study center. Following a 6-day washout period, participants then repeated the intervention using the other coffee.

2.4. Coffee preparation

Capsule coffee brand was meticulously selected due to the numerous plastic components within both the capsule and the machine that were likely to cause EC migration [13], as well as its popularity in the US. The capsule was composed of polystyrene and the water reservoir was composed of polystyrene. Capsule coffee was prepared from a single coffee brand. First, the brewing machine was run three times using only high-performance liquid chromatography (HPLC)-grade water to rinse any potential residue. Three capsules were then brewed sequentially using HPLC-grade water, and the resulting coffee was mixed (producing approximately 540 mL coffee). French press coffee was brewed using stainless steel equipment (French press, coffee bean grinder and water kettle) and a whole bean version of the same brand of coffee. First, the coffee grinder was filled with beans and ground for 14 s. Then, 27 g of

| Table 1 |
| List of optional food and beverage provided to participants. |
| Breakfast | Lunch |
| 1 protein bar | 1 protein bar |
| 12 fl oz. ginger ale | 12 fl oz. ginger ale |
| 1 medium banana | 1 medium banana |
| 1 medium apple | 1 medium apple |
| 1 slice bread | 1 slice bread |

Fig. 1. Randomized crossover intervention study design. Potential participants were screened and assessed for eligibility then randomized into either the capsule coffee or French press coffee group. After a 7-day run-in period, participants consumed their assigned coffee and provided urine samples. Following a 6-day washout period, each participant consumed the other coffee type and provided urine samples.
ground coffee (equivalent to the amount of coffee contained in three capsules) was placed in the French press along with 600 mL hot HPLC-grade water and brewed for 4 min producing approximately 540 mL coffee. Coffee was immediately served in a ceramic mug.

2.5. Urine collection

In order to minimize potential EC contamination from plastic containers, urine was collected in 2 L glass amber bottles, with aluminum foil wrapped underneath the cap. Canvas bags were provided for ease of transportation and privacy. In total, for each coffee type, three separate urine samples were collected over 24 h: one at baseline immediately prior to coffee consumption, one for 6 hours following coffee consumption, and one for 18 h that followed. Therefore, each participant contributed with 6 urine samples, which were aliquoted into ultra-low temperature-resistant glass vials and stored in an ultra-low temperature freezer immediately until analysis.

2.6. EC analysis

The extraction, identification, and quantification of ECs from coffee and urine samples have previously been described [13]. Briefly, contents of ECs in samples were identified and quantified using ultra-performance liquid chromatography with tandem mass spectrometry (UPLC-MS/MS). Unknown ECs were identified and characterized via retention time and m/z values of compounds and fragments, or signature ion fragments of a peak generated by EC standards. The ECs targeted for analysis were caprolactam, BPA, BPF, BPS, benzophenone (BP), 4-nonylphenol (4-NP), dimethyl terephthalate (DMTP), dibutyl phthalate (DBP), and di(2-ethylhexyl) phthalate (DEHP). Method validation, UPLC and mass spectrometry conditions are outlined in a previous study [13].

2.7. Data analysis

To summarize the EC content of urine samples, the number of positive samples and maximum values were displayed. Given the low frequency of detectable ECs in our samples, it was not meaningful to calculate a measure of central tendency such as geometric mean, which is standard with a high frequency of positive samples [17–19].

3. Results

3.1. Baseline characteristics

Those characteristics of the participants at baseline are shown in Table 2. The majority were females, normal weight, and Caucasian. Asian-American/Pacific Islander was the second most common race/ethnicity, followed by Hispanic, African-American and other. The first reported place of coffee consumption was at home, followed by coffee brewery or restaurant, and lastly school or work. The most commonly consumed coffee was drip, followed by other coffee, espresso, and capsule.

3.2. EC analysis of coffee and urine

Of the nine ECs tested in coffee samples (BPF, DEHP, BPA, BPS, BP, 4-NP, DBP, caprolactam, and DMTP), only BPF and DEHP were detected in quantities above their respective limits of detection (0.75 ng/mL for BPF and 0.001 ng/mL for DEHP). Capsule coffee contained 16.1 ng/mL BPF and 1.12 ng/mL DEHP while French press coffee contained 13.9 ng/mL BPF. The same nine ECs were tested in all urine samples after consumption of capsule or French press coffee (Table 3). DEHP, BPA, 4-NP, BP, BPF and DBP were detected in urine samples, in decreasing order of frequency. Caprolactam, BPS and DMTP were not detected in any urine samples. Overall, detection frequencies were very low, ranging from 0% to 10.56% of urine samples. DEHP and BPA were the two most common, with detection frequencies of 10.56% and 9.44% and max values of 23.10 and 209.85 ng/mL urine, respectively. Detection frequencies for 4-NP, BP, BPF and DBP were lower than those of DEHP and BPA, each ≤ 3.33%.

Comparisons of EC content in urine samples by coffee type and time is displayed in Fig. 2. As BPF and DEHP were the only two ECs detected, these were the primary ECs of interest. Only one urine sample contained BPF after consuming French press coffee (F24). No urine samples collected after consuming capsule coffee contained BPF. DEHP was detected in urine collected prior to consuming capsule coffee among five participants, one of whom also had a detectable amount of DEHP in the Cs urine sample. DEHP was also detected in five F5 and four F6 urine samples, though none were from the same participant. Only one F24 and one Cs sample contained DEHP. BPA was detected in three FB and six CB urine samples; one participant with BPA detected in FB also had it detected in F6. No other urine samples positive for BPA derived from the same participant. Notably, there were no instances in which the same EC was detected across coffee types (i.e., in both F5 and Cs or F24 and C24) within the same participant, which would have enabled direct comparisons between coffee types at the same time point.

4. Discussion

The objective of this study was to determine whether consuming capsule coffee leads to higher EC exposure compared to French press coffee in a human crossover intervention study. This was in response to the prevailing hypothesis that contact with plastic materials during and surrounding (such as handling or processing) the coffee brewing process would lead to greater EC contamination in coffee and, therefore, in excreted urine. To the best of our knowledge, this was the first human intervention study that compared urinary EC excretion between capsule and French press coffee. In respect to our hypothesis, there was not enough evidence to substantiate the notion that consuming capsule coffee increases urinary EC excretion compared to French press coffee despite its higher concentration and number of ECs. Importantly, this does not prove a lack of disparity, especially as the low prevalence of ECs in urine samples precluded the use of standard statistical comparisons; rather, it suggests that if there is indeed a difference in urinary EC excretion between the coffee types, a higher-powered study would be required to detect it. Furthermore, there was a low number of urine

| Variables | Participants |
|-----------|--------------|
| Female | 22 (73.3%) |
| Age (years) | 24.8 (5.5) |
| Race/ethnicity | | |
| Caucasian | 15 (50%) |
| Asian-American/Pacific Islander | 11 (36.7%) |
| Hispanic | 2 (6.7%) |
| African-American | 1 (3.3%) |
| Other | 1 (3.3%) |
| BMI (kg/m²) | 24.2 (4.1) |
| Usual location of coffee consumption | | |
| At home | 27 (73.0%) |
| At coffee brewery/restaurant | 6 (16.2%) |
| At school/work | 4 (10.8%) |
| Coffee consumption over the past 12 months (cups per day) | 1.2 (1.1) |
| Estimated lifetime frequency of consumption (times) | | |
| Drip coffee | 777.5 (2033.6) |
| Other coffee | 243.3 (346.8) |
| Espresso coffee | 202.5 (261.8) |
| Capsule coffee | 149.7 (459.9) |

Data are presented as n (%) for categorical and mean (SD) for continuous variables.

a Participants were allowed to select multiple locations.

b Median (IQR) presented due to extreme outliers.
The total amount of BPF present in 540 mL of coffee was 7.51 µg, indicating that ECs present in coffee may not appear in urine as expected. In other words, each coffee sample may have had a different content of ECs owing to possible differences in processing, shipping, storage or handling conditions. We attempted to account for this variation and increase representability by mixing three different capsules for each coffee sample rather than using a single capsule. In our previous analysis, we reported that the same brand of capsule coffee used in this study contained BPF but not DEHP (Capsule #1 from [6]), indicating that there are variations in the content of ECs even within the same brand of coffee. The source of BPF contamination for French press coffee was 0.61 µg for French press coffee (13.9 ng/mL multiplied by 540 mL coffee), far below a proposed intake limit of 0.778 µg for capsule coffee 0-hour (baseline); 1.85 µg for French press coffee 6-hour (hours 0–6); 12.75 µg for capsule coffee 24-hour (hours 0–24). For DEHP, the total amount in capsule coffee was 0.61 µg (1.12 ng/mL * 540 mL coffee), also lower than the tolerable daily intake of 50 µg/kg body weight/day [20].

Environmental contamination. Observational studies have reported the widespread detection of ECs such as BPA, BPS and DEHP in urine across various populations [17,18,23–25]. Common sources of ECs include canned food and beverages [14,15,26], coffee [4,5,13], ultra-processed food [27] and food packaged in plastic [12], but also include environmental sources such as thermal paper receipts [28,29], clothing made of synthetic polymers, and water sourced from areas near waste or sewage [30]. Even in a strictly controlled intervention study designed to reduce dietary exposure to DEHP [31], urinary DEHP concentrations unexpectedly became elevated. The authors determined that this was related to the high DEHP concentration in coriander, a herb that is typically consumed in very low amounts (relative to food/beverages), which suggests that even a seemingly inconsequential ingredient may contribute substantially to one’s total exposure. In our study, there were relatively higher occurrences of BPA and DEHP in urine, likely owing to their general ubiquity [18,19]. We expected that the concentrations of ECs (particularly DEHP and BPF) in 6-hour urine samples would be elevated relative to baseline after consuming coffee. While this indeed occurred for several participants, no participant had detectable amounts of DEHP or BPF in both the baseline and 6-hour samples except for DEHP in one participant. This serves as an important comparison because elevations in urine samples post coffee consumption may have occurred due to spurious or unknown EC exposure occurring outside of the study center.
lack of ECs in urine samples collected after drinking either type of coffee indicates that coffee is likely not a substantial contributor to EC exposure, at least via the two methods tested. The question as to whether there is a toxicokinetic difference in EC metabolism between capsule coffee and French press coffee remains unclear and requires further investigation.

There are several limitations noted in this study. First, we did not measure a urine sample prior to the run-in period, which prevents us from quantitatively assessing the degree to which the design for reducing external EC exposure actually reduced EC exposure. However, it does not affect the primary outcome, which was the comparison between samples of coffee types post-coffee consumption (i.e., between 6-hour urine samples). Second, only one brand of coffee was used in the intervention. The purpose of this was to eliminate the effect of the variability in EC content of other brands or flavors, but this limits external validity. Third, the intervention was short term and EC concentrations were measured after drinking only one 540 mL cup of coffee. In reality, coffee may be consumed multiple times daily and over many years. Thus, there may be cumulative effects of repeated coffee consumption on urinary EC exposure and subsequent biological effects.

Fig. 2. Scatter plot of individual participants’ concentrations of ECs in urine samples (n = 30). Zero-values are stacked. Colored/non-circle shaped points indicate positive samples originating from the same participant. C₀, capsule coffee 0-hour (baseline); C₆, capsule coffee 6-hour (hours 0–6); C₂₄, capsule coffee 24-hour (hours 6–24). F₀, French press coffee 0-hour (baseline); F₆, French press coffee 6-hour (hours 0–6); F₂₄, French press coffee 24-hour (hours 6–24). 4-NP, 4-nonylphenol; BP, benzophenone; BPA, bisphenol A; BPF, bisphenol F; DBP, dibutyl phthalate; DEHP, di(2-ethylhexyl) phthalate.
can realistically only be accounted for in decades-long observational studies. Finally, the relatively small sample size combined with the low detection frequencies of ECs meant that formal statistical tests of comparison were not appropriate. This also meant that the detection frequencies were very low and that the majority of urine samples were absent of ECs. There were also some notable strengths. First, the relatively low number of urine samples positive for ECs indicated that there was minimal contamination from sources other than the coffee, therefore giving a low degree of noise. Second, the crossover design limits inter-individual variation from potentially confounding variables that might affect EC exposure or metabolism such as one’s own metabolism, diet, and lifestyle habits, as each participant served as his/her own control. Finally, we assessed coffee and urine samples for a wide variety of ECs that have negative biological effects and are pervasive in the diet, many of which have not been previously assessed in this context.

In conclusion, in this 2-week crossover intervention, consuming capsule coffee did not lead to greater urinary EC excretion compared to consuming an identical brand of French press coffee. The low frequencies of ECs detected also indicate a low potential for harm on reproductive systems via EC contamination for either coffee preparation method. Future studies should broaden the types of coffee (other brands, other methods, etc.) evaluated, incorporate larger sample sizes, and investigate potential deleterious effects in physiological (primarily reproductive) systems.

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CRediT authorship contribution statement

**Junichi R. Sakaki:** Data curation, Formal analysis, Investigation, Writing – original draft. **Anthony A. Provatas:** Formal analysis, Investigation, Software, Validation, Writing – review & editing. **Christopher Perkins:** Formal analysis, Investigation, Software, Validation, Writing – review & editing. **Ock K. Chun:** Conceptualization, Data curation, Funding acquisition, Project administration, Resources, Supervision, Visualization, Writing – review & editing.

Declaration of Competing Interest
The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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