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Concentrations of Morphine and Codeine in Paired Oral Fluid and Urine Specimens Following Ingestion of a Poppy Seed Roll and Raw Poppy Seeds

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Interpretation of opiate drug test results can be challenging due to casual dietary consumption of poppy seeds, which may contain variable opiate content. Opiate concentrations in paired oral fluid (OF), collected with the Oral-Eze® Oral Fluid Collection System, and urine were analyzed after ingestion of poppy seeds from the same source, consumed raw or contained in a roll. In Part 1, 12 individuals consumed equal portions of a poppy seed roll. For Part 2, the same individuals consumed an equivalent quantity of raw poppy seeds, containing ∼3.2 mg of morphine and 0.6 mg of codeine. Specimens were analyzed both by enzyme immunoassay (opiates) and by GC–MS (morphine/codeine). Urinary morphine was between 155–1,408 (roll) and 294–4,213 ng/mL (raw), measured at 2, 4, 6 and 20 h post-ingestion. Urinary codeine concentrations between 140–194 (roll) and 121–664 ng/mL (raw) were observed up to 6 h post-ingestion. Following consumption of raw poppy seeds, OF specimens were positive, above LOQ, from 0.25 to 3.0 h with morphine ranging from 7 to 600 ng/mL and codeine from 8 to 112 ng/mL. After poppy seed roll consumption, morphine concentrations of 7–143 ng/mL were observed up to 1.5 h with codeine detected in only 5.5% of OF specimens and ranging from 8 to 28 ng/mL. Combined with the existing poppy seed literature, these results support previous findings and provide guidance for interpretation of OF opiate testing.

Introduction

Morphine and codeine are naturally occurring substituents of the poppy plant, *Papaver somniferum* (1). Since the 1980s, concerns regarding positive opiate drug tests following the ingestion of poppy seeds in bagels, pastries and food prepared according to various cultural traditions have been expressed (2–10). This has led to the so-called 'poppy seed defense' as an explanation for positive morphine and codeine findings in urine drug tests. Therefore, distinguishing between dietary poppy seed ingestion and legitimate versus non-prescribed opiate or heroin abuse is important for individuals performing safety-sensitive duties as well as other workers subject to routine drug testing for opiates. Previous studies have shown that ingestion of poppy seeds can result in urinary concentrations of morphine in excess of the established Department of Health and Human Services' (DHHS) Substance and Abuse Mental Health Services Administration (SAMHSA) drug-testing cutoff of 2,000 ng/mL [see Lachenmeier *et al.* (11)]. The opiate content of poppy seeds varies greatly and is dependent on the seed origin and method of processing, with documented morphine concentrations ranging from 0.1 to 294 μg/g (4–6, 9, 12, 13). To minimize the number of positive opiate tests resulting from poppy seed consumption (i.e., 'incidental exposure' from food products), SAMHSA raised the federally mandated cutoff concentration for morphine and codeine from 300 to 2,000 ng/mL in November 1998 (14).

Urine has historically been used in both federally regulated and non-regulated (company policy) workplace drug testing. However, oral fluid (OF) is also increasingly being used in clinical and non-regulated forensic drug testing settings. OF is a suitable alternative specimen due to its ease of collection, difficulty adulterating or substituting and detection window which better reflects potential impairment or more recent drug use (15–17). In July 2012, SAMHSA's Drug Testing Advisory Board (DTAB), a scientific council which advises SAMHSA's Federal workplace drug-testing program, issued recommendations to evaluate OF as an approved alternative specimen for federally regulated workplace drug testing programs.

To date, there are limited studies in OF using commercially available collection devices to evaluate the impact of consumption of poppy seed-containing products (bagel, cakes, rolls, etc.) on opiate test results. Detection of morphine and codeine subsequent to poppy seed exposure has been explored primarily in urine (2, 3, 6, 9, 11, 13, 18, 19) and serum (3, 13, 20); yet fewer studies have been published in alternative matrices such as hair (21) and OF (22–24). The purpose of the present study is to investigate opiate analytical results in paired urine and OF specimens, following ingestion of poppy seeds of known opiate content, using the Oral-Eze® Oral Fluid Collection System and the CEDIA® Opiate OFT Assay, an FDA-cleared collection and testing system, and GC–MS testing. Morphine and codeine concentrations were monitored in urine and OF collected from participants after consumption of a Ukrainian-style poppy seed roll and raw poppy seeds in a two-part study.

Materials and methods

Study design

Twelve (seven male/five female) healthy volunteers, 26–64 years old and weighing 49.0–88.6 kg participated in a two-part study. In the first part of the study each participant consumed one traditionally baked Ukrainian-style poppy seed roll, which was prepared with ∼15 g of poppy seeds per serving. During preparation, the poppy seeds were rinsed using room temperature water, then soaked in hot water (>80°C) to allow for seed expansion. The swollen seeds were ground with recipe ingredients (nuts, sugar, etc.), spread on flattened dough and formed. The rolls were allowed to rise for 3–4 h (25–35°C) prior to baking for 35–50 min at 350°F (175°C). For the second part of the study, 2 days later, participants ingested 15 g of raw poppy seeds from the same source used to prepare the rolls. Three participants completed Part 2 within 7 days from Part 1.

All study volunteers provided written informed consent to Quest Diagnostics Incorporated to participate in the research study. Each participant was assigned a unique coded identifier, which was used to document the date and time on the custody and control form used during specimen collection for Parts 1 and 2 of the study. Baseline (pre-ingestion) OF and urine specimens were collected up to an hour before the start of each part of the study. Participants were permitted to drink up to 180 mL of fluid (evenly divided, where possible) during the first 2 h, then *ad libitum* for the remainder of each part of the study. Participants were instructed to consume the poppy seeds within 15 min and were permitted to drink during this time; OF collection began 15 min after the completion of poppy seed ingestion. OF specimens were collected at 0.25, 0.5, 1, 1.5, 2 and 3 h after poppy seed ingestion (baked roll and raw) and also at 4, 5 and 6 h post-ingestion. Urine was collected at 2, 4 and 6 h after ingestion. Approximately 20 h (upon arising the following morning) after poppy seed ingestion, the last OF and urine void was obtained.

Specimen testing

Coded specimens were transported, accessioned and processed for routine drug testing at Quest Diagnostics Laboratory (Lenexa, KS, USA). All OF and urine specimens collected were screened by enzyme immunoassay and confirmed for morphine and codeine via gas chromatography–mass spectrometry (GC–MS), using previously validated methods established by the NLCP certified and CAP-FDT accredited toxicology laboratory.

Poppy seed opiate concentration and serving size

Poppy seed concentrations of morphine and codeine were determined by sonication of methanolic extracts containing 0.1% acetic acid for 2 h (25). Quantitative values obtained by GC–MS analysis of poppy seed aliquots were run in duplicate using three representative samples from the same source of seeds used during the study ($n = 6$), which were purchased from City Market (Kansas City, MO, USA). The poppy seeds contained a mean concentration of 219.6 μg/g of morphine and 39.3 μg/g of codeine, with coefficients of variation calculated at 8.2 and 6.1%, respectively. In Part 1, individual participants consumed a roll containing 14.58 g (dry weight before preparation) of poppy seeds per serving, for a total of 3.2 mg of morphine and 0.6 mg of codeine. In Part 2, the total serving of morphine was 3.3 mg and codeine was 0.6 mg after consumption of 15 g of raw poppy seeds.

Immunoassay

Initial testing for OF and urine was performed on a 5400 Beckman Coulter automated chemistry analyzer (Beckman Coulter, Brea, CA, USA) with methods validated for workplace drug testing analyses, using controls targeted at ±25 and ±50% of cutoff concentration for urine and OF, respectively. For OF screening, morphine and codeine concentrations were determined by β-galactosidase recombinant DNA technology using Thermo Scientific CEDIA® Opiate OFT Assay (Thermo Fisher Scientific, Fremont, CA, USA), implementing a 30 ng/mL cutoff in neat OF. Drug concentrations in urine were determined using the DRI® Opiate Assay (Thermo Fisher Scientific), which utilizes a decision point of 2,000 ng/mL for opiates (morphine).

Measurement of creatinine

Creatinine concentration was determined using a kinetically modified Jaffe procedure (Beckman Coulter Urine TOX reagent), with the rate of change proportional to creatinine concentration, measured bichromatically at 520 and 800 nm wavelengths. Urinary drug concentrations are expressed as raw quantitative values or normalized to 100 mg/dL of creatinine and reported as ng of drug per 100 mg of creatinine, designated as $Creat_{100}$. Urine samples were normalized to creatinine to minimize the impact of urine dilution effects on drug concentrations.

Sample preparation and GC–MS

Urine

All collected samples, whether the initial screening was presumptive positive or negative, were quantified for morphine and codeine by GC–MS using a cutoff concentration of 2,000 ng/mL. Each calibrator, quality control (QC) and participant sample contained a final concentration of 2,000 ng/mL of morphine and codeine deuterated internal standards (ISTDs). To 1 mL of urine, 2 mL of 2.0 M sodium acetate buffer (pH 5.0) was added prior to the addition of 100 μL of β-glucuronidase (Campbell Science, Rockford, IL, USA). Samples were hydrolyzed for 2.5 h at 60°C. Three milliliters of 0.1 M phosphate buffer (pH 6.0) was added to the samples, which were centrifuged (2,500 × *g*), then extracted using UCT Clean Screen™ DAU Solid Phase Extraction (SPE) Columns (Waltham, MA, USA) and eluted with methylene chloride : isopropyl alcohol (80 : 20, v/v) containing 2% ammonium hydroxide. Eluates were concentrated under nitrogen and derivatized with *N*-methyl-bis(trifluoroacetamide).

Quantitative testing was performed on an Agilent 5975C gas chromatograph–mass spectrometer utilizing previously validated analytical methods. For each confirmation run, calibration was achieved using a single-point calibrator, target concentration of 2,000 ng/mL, for both morphine and codeine. Negative, 40 and 125% cutoff QC samples were analyzed with each analytical run. Chromatographic separation was achieved with a 3.0 μg/mL injection of extracts (split 40 : 1) on a 5% phenylmethyl silicone DB5 cross-linked capillary column (15 m, 0.25 mm; Agilent Technologies, Santa Clara, CA, USA) with an inlet temperature of 280°C and gradient temperature programing. Extracts were monitored in SIM mode at (quantification ions in bold) m/z **364**, 477 and 311 for morphine (m/z **367**, 480 morphine-d_3) and m/z **282**, 395 and 283 for codeine (m/z **288**, 401 codeine-d_6). GC/MSD Chemstation software (Agilent Technologies) was used for data processing. Extracts were considered acceptable if the peaks were symmetrical with valid (±20%) ion ratios, both 40 and 125% control were within ±20% of the established range, negative control(s) had a quantitative value less than the limit of detection (LOD) and peaks were within 2% of the retention time standard.

Oral fluid

OF was collected using the Oral-Eze Oral Fluid Collection system, which implements a 3-fold dilution of original (neat) OF with buffer preservative solution contained in the collection/transport tube. All participant samples, whether the initial screening was presumptive positive or negative, were quantified for morphine and codeine by a previously validated GC–MS

method using a cutoff concentration of 30 ng/mL (neat OF equivalent). Morphine and codeine deuterated ISTDs were added to each calibrator, QC and participant sample to achieve a final concentration of 39.9 ng/mL (equivalent concentration in neat OF). One milliliter of 0.1 M sodium acetate buffer (pH 4.5) and 500 μL of 10% methoxamine were added to each 300 μL aliquot of calibrator, QC and participant sample. After 1 h incubation, samples were extracted with CEREX Trace B solid phase extraction columns (SPEware, CA, USA) and eluted with ethyl acetate containing 2% ammonium hydroxide. Eluates were evaporated to dryness and derivatized using *N,O*-Bis (trimethylsilyl)trifluoroacetamide containing 1% trimethylchlorosilane. Extracts were injected onto a 6890N Agilent GC System equipped with a Deans Switch and coupled to a 5975 Agilent Mass Selective Detector using negative chemical ionization and utilizing deuterated ISTDs for retention time and quantification reference. Calibration was performed for each confirmation run using a single-point calibrator, target concentration of 30 ng/mL, for both morphine and codeine. Negative, 50 and 125% cutoff QC samples were included with each analytical run.

Chromatographic separation was achieved with a 5.0 μL injection volume (pulsed splitless) on column 1 consisting of a DB-1 column (15 m, 0.25 mm, 0.25 μm; Agilent Technologies) with an inlet temperature of 250°C and a pulse pressure of 40.0 psi and column 2 consisting of a DB-17 column (15 m, 0.25 mm, 0.25 μm; Agilent Technologies) using ultra-pure helium carrier gas. SIM acquisition mode was used to monitor the following ions (quantitation ions in bold): m/z**429**, 430, 401 for morphine (m/z**435**, 420 morphine-d_6) and m/z**371**, 343, 372 for codeine (m/z**377**, 349 codeine-d_6). GC/MSD Chemstation software was used for data acquisition, and extracts were considered acceptable if peaks were symmetrical, the negative control was less than the LOD, both 50 and 125% cutoff QC controls were within ±25% of the established mean and peaks were symmetrical with valid (±20%) ion ratios within 2% of the retention time standard.

Data analysis

Data were analyzed and graphed using the Microsoft® Excel (Microsoft Office Profession Plus 2010) and the GraphPad® Prism statistical software (Version 5.0, La Jolla, CA, USA). Standard deviations given in Table I were calculated using sample ($n - 1$) standard deviation.

Results

Poppy seeds were obtained from a local spice market, analyzed and determined to contain 3.2 mg of morphine and 0.6 mg of codeine per 15 g (dry weight) portion of poppy seeds. For Part 1, participants consumed equal servings of a traditional Ukrainian-style poppy seed roll prepared with ~15 mg of poppy seeds per serving. Part 1 of the study (roll consumption) was started on experimental Day 1, with Part 2 (raw poppy seed ingestion) performed on the second day (i.e., Day 3) after the Part 1, with the exception of the three participants referenced in the Study Design section above. Urine and OF specimens confirmed by GC–MS at or above the cutoff of 2,000 ng/mL (urine) and 30 ng/mL (OF) were qualitatively positive by enzyme immunoassay. All participants' baseline (pre-ingestion) urine and OF specimens (Studies 1 and 2) were negative.

Urine specimens

Urine specimens were analyzed by enzyme immunoassay for amphetamines (1,000 ng/mL), cocaine metabolites (300 ng/mL), THC (50 ng/mL), opiates (2,000 ng/mL) and phencyclidine (25 ng/mL) with confirmation of morphine, codeine, hydromorphone, hydrocodone and 6-acetylmorphine performed by GC–MS with a limit of quantitation (LOQ) of 75 ng/mL for morphine and codeine. A total of 120 urine specimens were collected for baseline testing and post-ingestion analysis at 2, 4, 6 and 20 h. Overall, 95.8% (46/48) of specimens were positive for morphine, with 6.2% (3/48) of specimens positive for codeine at or above the LOQ in Part 1 (roll) of the study. In comparison, 100% of samples were morphine-positive and 47.9% codeine-positive after Part 2 (seeds). Table I provides the mean and range of raw urinary opiate concentrations, along with standard deviation and sample size for each time point after roll and raw poppy seed ingestion. Morphine C_{min} was 155 ng/mL (6 h) with C_{max} at 1,408 ng/mL (2 h) after consumption of the poppy seed roll. Codeine C_{min} was 140 ng/mL (4 h) with a C_{max} of 194 ng/mL (4 h). After raw poppy seed ingestion, the C_{min} was 188 ng/mL for morphine and 121 ng/mL for codeine, at 2 and 6 h, respectively.

Table I
Urine and Neat OF Concentrations (ng/mL) of Morphine and Codeine after a Single Serving of Raw Poppy Seeds or Baked in a Roll

Time (h)	Morphine		Codeine	
	Roll	Seeds	Roll	Seeds
Urine				
2	**539** (188–1,408), 343, *n = 12*	**1,360** (188–3,017), 977, *n = 12*	162, *n = 1*	**315** (135–501), 164, *n = 5*
4	**697** (258–1,356), 302, *n = 12*	**1,743** (399–4,213), 1,123, *n = 12*	**167** (140–194), 38, *n = 12*	**313** (149–664), 159, *n = 9*
6	**461** (155–954), 222, *n = 12*	**1,546** (294–3,622), 1,067, *n = 12*	ND, *n = 11*	**275** (121–479), 125, *n = 9*
20	**348** (183–471), 121, *n = 10*	**553** (316–910), 179, *n = 12*	ND, *n = 12*	ND, *n = 12*
OF				
0.25	**35** (7–143), 37, *n = 11*	**158** (47–284), 91, *n = 11*	**18** (9–28), 13, *n = 2*	**49** (16–112), 32, *n = 11*
0.5	**18** (12–37), 12, *n* = 4	**85** (19–333), 86, *n = 11*	8, *n = 1*	**23** (9–59), 15, *n = 11*
1	**11** (8–18), 4, *n = 4*	**30** (9–94), 25, *n = 10*	ND, *n = 12*	**14** (10–22), 4, *n = 7*
1.5	**16** (8–25), 12, *n = 2*	**29** (9–83), 25, *n = 8*	8, *n = 1*	**13** (8–19), 7, *n = 5*
2	ND, *n = 12*	**15** (8–30), 8, *n = 6*	ND, *n = 12*	**9** (8–12), 2, *n = 3*
3	ND, *n = 12*	**10** (7–18), 5, *n = 4*	ND, *n = 12*	ND, *n = 12*

Values represent the **mean** (range), SD, *sample size*.
ND, not detected.

C_{max} after raw poppy seeds was 4,213 ng/mL for morphine (4 h) and 664 ng/mL for codeine (4 h). The percent of morphine and codeine-positive urine specimens at each collection time and cutoff level are shown in Table II. Based on the GC–MS testing and using a 300 ng/mL cutoff, 75% (36/48) of the total specimens collected at 2, 4, 6 and 20 h after Part 1 (roll) contained morphine concentrations above cutoff, whereas no specimens were positive for morphine when a 2,000 ng/mL cutoff was utilized. In Part 2 (raw), depending on the time after collection, up to 100% of specimens were positive for morphine (300 ng/mL), with 33.3% of specimens positive at 2 h (2,000 ng/mL). Conversely, 66.7% of specimens were codeine-positive 4 h after raw poppy seed ingestion. Figure 1 depicts normalized urinary concentrations of morphine and codeine after poppy seed roll or raw poppy seed ingestion with 300 and 2,000 ng/mL cutoffs represented (dashed lines). Using a 300 ng/mL cutoff, morphine concentrations after roll consumption were above cutoff at 2, 4, and 6 h, while codeine concentrations remained below cutoff at every time point. After raw poppy seeds, morphine concentration exceeded the 300 ng/mL threshold from 2–20 h, with codeine concentrations above the cutoff at 2 and 4 h. Following raw poppy seed ingestion, morphine concentrations were greater than the 2,000 ng/mL cutoff at 2 and 4 h, with codeine concentrations below the threshold across experimental time points.

OF specimens

OF specimens were analyzed by enzyme immunoassay for methamphetamines (120 ng/mL), amphetamine (150 ng/mL), cocaine metabolites (15 ng/mL), marijuana (parent-THC) (3 ng/mL), opiates (30 ng/mL) and phencyclidine (3 ng/mL) with confirmation of morphine, codeine, hydrocodone and hydromorphone performed by GC–MS operating with a LOQ of 7.5 ng/mL for morphine and codeine. OF was collected prior to the start of each study and at 0.25, 0.5, 1, 1.5, 2, 3, 4, 5, 6 and 20 h after poppy seed ingestion. Since no OF specimens in Part 1 or 2 were positive for morphine or codeine after 3 h, data collected at 4, 5, 6 and 20 h were excluded from the final analyses. A total of 246 OF specimens were collected using the Oral-Eze device. Of those, analytical results after poppy seed ingestion were not obtained from Participant 102 at 1.5 h and Participant 106 at 1 h. Based on the GC–MS testing, 20.8% (15/72) of specimens were positive for morphine and 1.3% (1/72) were positive for codeine at or above method LOQ for study Part 1 (roll). In Part 2 (seeds), 61.4% (43/70) of samples were morphine-positive with 45.7% (32/70) codeine-positive at or above the LOQ. OF mean and range concentrations (ng/mL), along with standard deviation and sample size for each time point, are summarized in Table I. After the roll, morphine C_{min} was 7 ng/mL with a C_{max} of 143 ng/mL, both observed at 0.25 h. Codeine C_{min} was 8 ng/mL (0.25 and 1.5 h) with a C_{max} of 28 ng/mL observed at 0.25 h. Following raw poppy seed ingestion, specimens exhibited a morphine C_{min} of 7 ng/mL (3 h) and a C_{max} of >600 ng/mL (0.25 h; data not shown), with the second highest concentration of 333 ng/mL observed at 0.5 h. Furthermore, codeine C_{min} was 8 ng/mL (1.5 and 2 h), with a C_{max} of 112 ng/mL at 0.25 h.

Table II summarizes morphine and codeine concentrations after poppy seed roll or raw poppy seed ingestion relative to a 30 ng/mL (neat OF) cutoff, illustrated by a dashed line in Figure 2. After poppy seed roll consumption, positivity, near

Table II

Percentage of Morphine- and Codeine-Positive Urine[a] and OF Specimens by Cutoff Level and Collection Time[b]

Urine	Morphine			
	300 ng/mL cutoff		2,000 ng/mL cutoff	
Time (h)	Roll	Seeds	Roll	Seeds
2	83.3	100.0	0.0	33.3
4	91.6	100.0	0.0	25.0
6	75.0	91.6	0.0	25.0
20	50.0	100.0	0.0	0.0
Urine	Codeine			
	300 ng/mL cutoff		2,000 ng/mL cutoff	
2	0.0	41.6	0.0	0.0
4	0.0	66.7	0.0	0.0
6	0.0	25.0	0.0	0.0
20	0.0	0.0	0.0	0.0
OF	Morphine		Codeine	
	30 ng/mL cutoff		30 ng/mL cutoff	
0.25	33.3	91.6	0.0	58.3
0.5	8.3	83.3	0.0	25.0
1	0.0	27.2[c]	0.0	0.0[c]
1.5	0.0	27.2[c]	0.0	0.0[c]
2	0.0	8.3	0.0	0.0
3	0.0	0.0	0.0	0.0

[a]Based on raw urinary drug concentrations.
[b]Percentage of the total specimens ($n = 12$) above designated cutoff.
[c]Total specimens ($n = 11$).

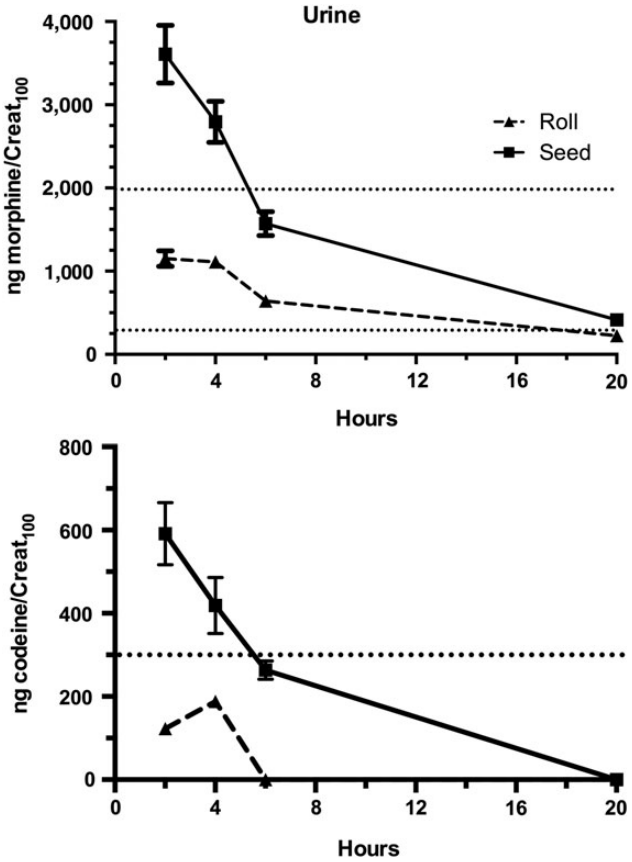

Figure 1. Morphine and codeine mean (±SEM) urinary concentrations normalized to creatinine after consumption of a poppy seed roll (roll) and raw poppy seeds (seed).

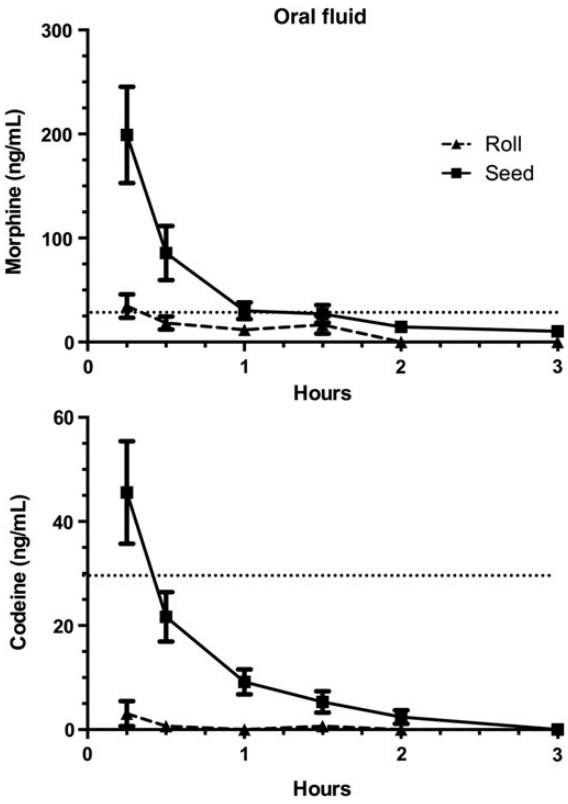

Figure 2. Mean (±SEM) morphine and codeine concentrations in OF after consumption of a poppy seed roll (roll) and raw poppy seeds (seed).

cutoff, was observed for morphine at 0.25 h, with concentrations falling below the cutoff from 0.5 to 3 h. No specimens collected after Part 1 (roll) were above cutoff for codeine across all experimental time points. Following raw poppy seed ingestion, morphine concentrations were greater than 30 ng/mL at 0.25 and 0.5 h, borderline positive at 1 and 1.5 h, and below cutoff at 2 and 3 h. In comparison, OF specimens were positive for codeine at 0.25 h, with decreasing concentrations observed across the study. At 3 h post-ingestion, no specimens contained detectable amounts of codeine. The percentage of morphine and codeine-positive OF specimens at each collection time is presented in Table II. In Part 1 (roll), 33.3% of specimens were positive for morphine 0.25 h after consumption, with no other specimens positive for morphine or codeine after 0.5 h. In Part 2 (raw), 91.6% of OF specimens were morphine-positive at 0.25 h, with positivity inversely proportional to post-ingestion time. In addition, 25 and 58.3% of specimens were positive for codeine after 0.25 and 1 h, respectively.

Discussion

The goal of this study was to investigate differences in morphine and codeine concentrations after ingestion of poppy seeds from the same source, consumed raw or contained in a traditionally prepared Ukrainian-style poppy seed roll. The morphine content in poppy seeds from around the world is variable (0.1–294 μg/g), and exposure concentrations depend on poppy seed origin, harvesting procedure and the method of poppy seed foodstuffs preparation (3–6, 9, 12, 13, 20, 21). Significant reductions in opiate content (≤80–90%) have been documented after food preparative processes, with decreased drug concentration shown after washing, soaking, grinding and baking (4, 5, 11, 25). In fact, complete removal of detectable concentrations of morphine was reported after commercial preparation (11). A limitation of this study is that morphine and codeine loss in the baked poppy seed roll was not quantified as part of this study; however, the results provided in Tables I and II demonstrate how processing of poppy seeds in the roll greatly reduced the percentage of urine (cutoff 2,000 ng/mL) and OF (cutoff 30 ng/mL) positivity. As previously discussed (2, 11), poppy seed studies have often failed to document the opiate content of poppy seeds used or have modeled a 'worst-case' scenario using large doses of raw poppy seeds. This study attempts to address such concerns by using the same poppy seeds and participants for both raw and prepared seeds, which permits comparison of opiate concentrations between studies. While anecdotal in nature, bolus ingestion of raw poppy seeds has been described as unpleasant, having little palatability (6, 21). Moreover, during our study, participants felt that 15 g of poppy seeds was close to the maximum tolerable limit of ingestion, which corresponds roughly to *ad libitum* ingestion by three volunteers in a study published by Rohrig and Moore (23). While people may use the so-called 'poppy seed defense', the quantity of poppy seeds used in previous studies are much larger than in diets that consist of casual exposure to poppy seed-containing foods (bagels, cakes, curries, etc.).

Our results demonstrate that consumption of poppy seeds in a Ukrainian-style roll versus raw poppy seed resulted in urinary morphine concentrations below 2,000 ng/mL as given in Tables I and II. In one investigation, a physician demonstrated that a typical poppy seed bagel produces urine concentrations of 336 and 446 ng/mL after 2 and 5 h of consumption, respectively (26). Conversely, Mule and Casella (10) described morphine concentrations in hydrolyzed urine in excess of 2,000 ng/mL after two poppy seed bagels. After eating a curry dish prepared with 25 g of poppy seeds (1,002 μg morphine/479.5 μg codeine), maximal urine concentration was 1,270 ng/mL (4). Furthermore, other publications report morphine urinary concentrations below the 2,000 ng/mL cutoff, following consumption of poppy seed-containing cakes (5, 9) and rolls (19). Our study revealed concentrations of morphine above the 2,000 ng/mL cutoff in urine specimens at 2, 4 and/or 6 h following ingestion of raw poppy seeds (Tables I and II). Taking into account potential dilution effects, specimens remained positive (2 and 4 h) after urine specimens were normalized to creatinine (Figure 1). Similar to findings of Smith *et al.*, after raw poppy seeds, no specimens were positive for codeine at the 2,000 ng/mL cutoff, despite an approximate 5-fold difference in total codeine dose between studies (0.6 versus 3.1 mg). Previous studies have documented urinary morphine concentrations above 2,000 ng/mL after ingestion of 9–21 g (23) and 40 g (63 ± 15 μg/g of morphine) of raw poppy seeds (3). After ingestion of a poppy seed cake containing 2.4–7.7 mg morphine (151.6 μg/g morphine), 37.5% (30/80) of urine specimens were >2,000 ng/mL (2–21 h post-ingestion), with a morphine concentration of 10,040 ng/mL (6 h post-ingestion) in one volunteer (12).

After raw poppy seed ingestion, OF specimens had morphine and codeine concentrations of 7–333 and 8–112 ng/mL,

respectively (Table I and Figure 2). These data are consistent with Niedbala *et al.* (22) who demonstrated positive opiate immunoassay results 15 min following consumption of up to a 40-g dose of commercially available, uncooked poppy seeds. Furthermore, Rohrig and Moore (23) detected morphine in OF up to 1 h after combined ingestion of a poppy seed bagel and canned poppy seed filling, with the total amount ingested ranging from 9.82 to 20.82 g. The maximum concentration of morphine detected in this study was 205 ng/mL with the OF_{max} occurring 15 min post-ingestion (23), which is consistent with our data of C_{max} of 284 ng/mL at 0.25 h (Table I). Most recently, Conchiero *et al.* published a study in OF using poppy seeds whereby the opiate content was quantified prior to ingestion. Subjects received two equal doses of raw poppy seeds 8 h apart, for a total ingested dose of 31.4 mg of morphine and 6.2 mg of codeine (24). After the first dose, peak morphine and codeine concentrations were 177 and 32.6 ng/mL, reported from 0.5 to 1 h and 0.5 to 2 h, respectively (24). Differences in maximum morphine (333 ng/mL) and codeine (112 ng/mL) concentrations from our study, compared with Conchiero *et al.*, may be explained by differences in ingestion, route of administration (raw versus suspension) and time after first collection (0.5 versus 0.25 h). Furthermore, positivity rates were more pronounced in both frequency and duration (Table II) after raw poppy seed ingestion (Part 2) when compared with consumption of the poppy seed roll (Part 1). While participants were permitted to drink during ingestion of poppy seeds, additional measures (rinsing oral cavity or brushing) were not performed prior to the start of OF collection(s). As a result, the initial morphine and codeine concentrations observed after raw poppy seed ingestion are likely affected by poppy seed residue in the oral cavity. In one study, opiate concentrations of 22 and 24 ng/mL were documented after ingestion of a poppy seed muffin by two volunteers; after the oral cavity was rinsed with mouthwash, opiate value was $\leq$12 ng/mL (27). Consequently, it cannot be determined in this study whether drug concentrations are wholly reflective of the systemic circulation.

After consumption of a poppy seed-containing roll, morphine concentrations of 7–143 ng/mL were observed up to 1.5 h post-ingestion, with codeine detected in only 5.5% of OF specimens. Importantly, if an opiate cutoff of 30 ng/mL is considered, consumption of a poppy seed roll does not produce a positive OF result 1–3 h after ingestion, with only one specimen observed above cutoff at 0.5 h (Table II). Thirty-three percent (4/12) of OF specimens were positive 0.25 h post-ingestion at this cutoff; however, individuals are routinely instructed to refrain from eating for at least 10 min prior to OF collection. Lastly, OF specimens were below the proposed cutoff (30 ng/mL) at 1 h, indicating there is a decreased likelihood of a positive morphine result in OF versus urine after bolus ingestion of raw poppy seeds.

Conclusions

OF drug testing is advantageous because collection is noninvasive, can be performed outside traditional testing facilities without gender-specific personnel requirements, is easily observed and is not easily adulterated or substituted. Morphine and codeine are detected in OF for 2 h after raw poppy seed ingestion, and only 0.5 h after consumption of a poppy seed-containing roll. These data suggest that OF, due to its narrower window of detection, may be a specimen less susceptible to the 'poppy seed defense' after casual dietary poppy seed consumption.

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Conflict of interest

Oral-Eze is a registered trademark of Quest Diagnostics Incorporated. All the authors are employees of Quest Diagnostics.

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