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Article

Elevated Urine Zinc Concentration Reduces the Detection of Methamphetamine, Cocaine, THC and Opiates in Urine by EMIT

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Methods for circumventing positive drug tests continue to evolve and are often spread through internet websites reporting on the proposed effectiveness of various adulteration methods. Recent claims of the use of zinc added directly to urine or ingested prior to urine collection have prompted investigation into the vulnerability of ELISA-based testing, providing interesting but inconclusive results. We investigated the potential interference of zinc used as a direct adulterant and after zinc self-administration for enzyme multiplied immunoassay technique (EMIT)-based drug abuse testing in urine. Negative urine samples and samples collected before and after zinc self-administration were fortified with *d*-methamphetamine, benzoylecgonine, morphine and 11-nor-9-carboxy-tetrahydrocannabinol prior to analysis by the EMIT. Our data indicate that zinc added directly to urine in concentrations 5,000 times higher than a typical random urine total zinc concentration is capable of producing false-negative results; however, self-administration of oral zinc was unable to generate random urine total zinc concentrations in the required range. Further, no evidence of a secondary interfering substance was observed as a result of oral zinc self-administration. Our results indicate that the total zinc concentrations required to directly interfere with EMIT-based testing are easily distinguishable from routine random urine total zinc concentrations, and that alleged oral ingestion of zinc does not produce total zinc concentrations capable of direct interference.

Introduction

The volume of drug abuse testing has grown considerably over the last decade. Inherent to the increase in testing is the development and propagation of novel methods for circumventing the generation of a positive drug-screening result. At the center of the struggle between testing and circumvention are websites providing a forum for discussion regarding drug-ingestion experiences, with a considerable amount of information instructing readers in methods proposed to aid in the generation of negative drug-screening results in spite of current drug use.

A recent report in the *Journal of Analytical Toxicology* aimed to test one claim regarding the use of zinc as an effective adulterant to evade a positive drug-of-abuse test result (1). Although this study was informative, the central hypothesis that a false-negative result was due to elevated zinc concentrations in the urine after zinc self-administration was untested, as no zinc concentrations in the urine were reported in this study. The published literature contains numerous studies on the excretion kinetics of zinc ingested through multiple means; however, no relevant literature exists regarding the excretion kinetics of zinc after the proposed method of zinc ingestion. Further, the peak zinc concentrations expected in the urine of patients self-administering zinc as a method to circumvent drug testing are unknown, and no conclusive cause-and-effect relationship can yet be inferred. Three testable hypotheses are proposed for the observations regarding zinc ingestion and immunoassay drug screening: (i) acute zinc ingestion as previously described results in zinc urine concentrations capable of directly interfering with immunoassay-based testing, (ii) a secondary substance is excreted as a result of zinc ingestion that interferes with immunoassay-based testing and (iii) zinc ingestion interferes with drug excretion, resulting in a negative immunoassay-based screen. The goals of this study were to determine whether enzyme multiplied immunoassay technique (EMIT)-based testing was subject to the interference described previously for ELISA-based testing, to investigate the expected concentration of zinc in the urine of patients after self-administration of zinc and to determine whether a secondary interfering substance was present as a result of zinc self-administration.

Materials and methods

Use of clinical samples and human subjects

This project and its protocols were approved by the University of Utah Institutional Review Board (protocol #7275 and #00054713).

Instrumentation

EMIT testing was performed on an Olympus AU400e automated clinical analyzer with Emit® II plus assay kits from Dade Behring (Cupertino, CA, USA). Total zinc concentrations were measured using a Perkin-Elmer Sciex Elan 9000 and DRC II inductively coupled plasma-mass spectrometer (ICP-MS; PE Sciex, Shelton, NJ, USA). Urine creatinine was measured by Roche Modular P (Roche Diagnostic, Indianapolis, IN, USA). Total zinc concentrations represent the combined concentrations of chelated and free zinc measured as zinc m/z 64.

Reagents

Drug-free urine specimens containing low concentrations of total zinc were pooled and used as a matrix to create fortified samples. Syva® EMIT® control Level 5 (Dade Behring) containing 2,000 ng/mL of *d*-methamphetamine, 1,000 ng/mL of benzoylecgonine, 4,000 ng/mL of morphine and 200 ng/mL of 11-nor-9-carboxy-THC was used as a working stock solution for negative urine fortification. Working zinc sulfate monohydrate (36.4% zinc by weight) stocks (Sigma-Aldrich, St. Louis, MO,

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USA) were prepared in pooled drug-free urine (prepared in-house, ARUP Laboratories Reagent Laboratory, Salt Lake City, UT, USA), and total zinc concentration was verified by ICP-MS. Prepared zinc sulfate monohydrate and Syva® EMIT® control Level 5 solutions were added to six separate drug-free urine samples to achieve the same concentration of each drug but different concentrations of total zinc. The final concentrations of each drug were 400, 200, 800 and 40 ng/mL for *d*-methamphetamine, benzoylecgonine, morphine and 11-nor-9-carboxy-THC, respectively. Total zinc concentrations ranged from 0.5 to 50 mg/mL. Zinc gluconate for the ingestion study was from Nature's Bounty (Bohemia, NY, USA) and was 14.4% zinc by weight.

Zinc ingestion protocol

Urine samples were collected from 31 healthy volunteers, including 17 females and 14 males, ranging in age from 23 to 63 years. Urine specimens were collected for pre- and post-zinc self-administration according to the following study design:

Day 1

Each participant was provided 10 doses of 50 mg of zinc gluconate, two trace element-free urine collection containers, two trace element-free transport tubes and two labels with unique IDs (e.g., PR001 and PO001 for pre- and post-samples, respectively). Instructions for specimen collection were provided to ensure environmental contamination was minimized.

Day 2

Each participant collected a morning void upon arrival at work as the pre-administration urine sample. 4 × 50 mg of zinc gluconate was ingested at ∼8 pm the same day with food.

Day 3

Each participant ingested 4 × 50 mg of zinc gluconate upon waking with food, and provided a second morning void upon arrival at work as the post-administration urine sample.

Sample analysis

Total zinc concentrations in the pre- and post-zinc self-administration samples were determined by ICP-MS and are reported in μg/dL or μg/g of creatinine (μg/g CRT). Pre- and post-urine specimens were screened for common drugs of abuse by EMIT prior to fortification with the Level 5 Syva® EMIT® control solution. Data from EMIT testing were reported as normalized absorbance to calibrators prepared at the cutoff concentrations for the respective drugs (300 ng/mL of *d*-methamphetamine; 150 ng/mL of benzoylecgonine; 300 ng/mL of morphine and 20 ng/mL of 11-nor-9-carboxy-THC). Investigation of a secondary interfering substance was conducted using pooled pre- and post-zinc self-administration samples based on the total zinc concentration. A total of two pre-pool (9 and 39 μg/dL) and three post-pool (7, 53 and 137 μg/dL) were generated in addition to a drug-free negative urine pool. Each pre- and post-zinc self-administration pool was fortified with the drugs of interest at their respective cutoffs (300 ng/mL of *d*-methamphetamine; 150 ng/mL of benzoylecgonine; 300 ng/mL of morphine and 20 ng/mL of 11-nor-9-carboxy-THC).

Data analysis

Data analysis was conducted using Excel (Microsoft Corporation, Bellevue WA, USA) and the open-source statistical language R (2). Graphs were generated using the ggplot2 R graphics package (3) and adobe illustrator (Adobe Systems Incorporated, San Jose, CA, USA). Significance testing was performed in R, with a one-sided paired *t*-test for pre- and post-ingestion comparisons.

Results

Zinc interference with EMIT-based testing

Negative, relative absorbance values for benzoylecgonine, morphine and 11-nor-9-carboxy-THC fortified urines were generated in the presence of ≥5 mg/mL of zinc and ≥10 mg/mL of zinc for *d*-methamphetamine (Figure 1). Clinically, these negative values would typically be reported as negative for the presence of tested drugs or with a comment indicating the presence of an interfering substance and the inability to provide a qualitative result. These results confirm that the presence of zinc in mg/mL concentrations interferes with EMIT-based testing for drugs of abuse.

Zinc concentrations in urine after self-administration

A concentration of zinc in urine at 5 mg/mL (the lowest concentration of zinc found to interfere with EMIT-based testing) is ∼4,200 times higher than the upper end of the established reference interval (15–120 μg/dL) and is highly inconsistent with values seen in routine clinical testing (Figure 2A). Healthy volunteers were recruited through an IRB-approved study to determine if concentrations consistent with those found to interfere with EMIT-based testing could be achieved through self-administration protocols as reported on various drug websites. Statistically significant increases in post-ingestion samples were found for both random zinc (μg/dL) and creatinine normalized zinc (μg/g CRT) with *P*-values of <0.001 (Figure 2B and C). Summaries of the results from the zinc self-administration study and routine zinc testing are provided in Table I.

Absence of a secondary interfering substance after zinc self-administration

Because the excreted total zinc concentrations after self-administration were considerably less than those observed from the direct addition of zinc to the urine, we tested the hypothesis that a secondary interfering substance could be excreted as a result of zinc self-administration. No substantial difference was seen for pooled pre- or post-ingestion samples fortified with the drugs of interest (Figure 3), indicating the absence of a secondary substance capable of interfering with the EMIT tests.

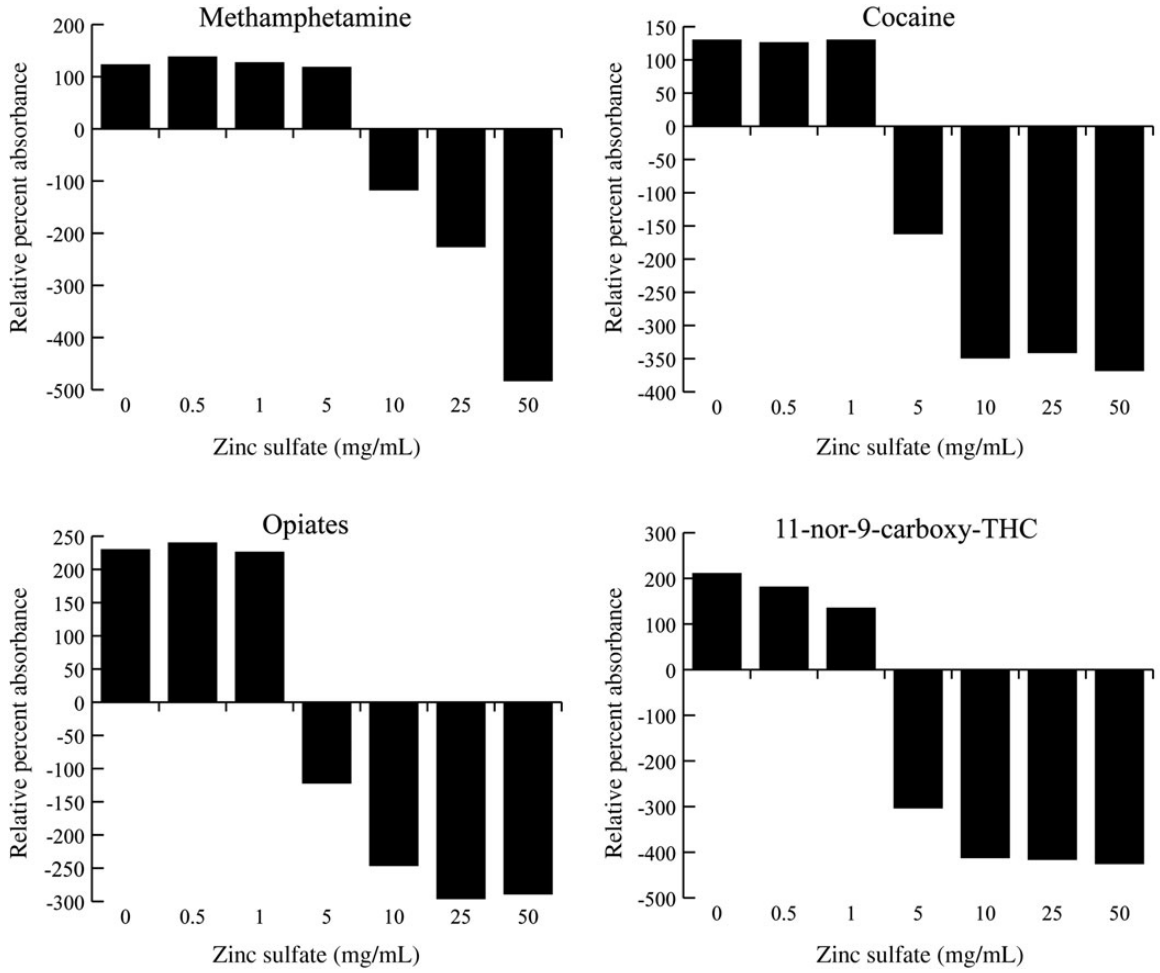

Figure 1. Relative absorbance for each drug class tested with increasing fortification of zinc sulfate. Negative urine was fortified at 400, 200, 800 and 40 ng/mL for *d*-methamphetamine, benzoylecgonine, morphine and 11-nor-9-carboxy-THC, respectively. All data were normalized to the absorbance of a prepared calibrator at the cutoff concentration for each assay (300 ng/mL of *d*-methamphetamine; 150 ng/mL of benzoylecgonine; 300 ng/mL of morphine and 20 ng/mL of 11-nor-9-carboxy-THC).

Discussion

The goals of this study were to determine whether EMIT-based testing was subject to the interference described previously for ELISA-based testing and to investigate the expected concentration of zinc in the urine of patients after self-administration of zinc in the manner consistent with reported attempts to circumvent positive drug-screening results. Our results show that (i) EMIT-based testing is susceptible to interference by zinc in concentrations in the mg/mL range and (ii) total zinc concentration in random urine after zinc self-administration ranged from 2 to 167 μg/dL.

Our first finding that concentrations of zinc in the mg/mL range produced false negatives for EMIT-based testing was observed for *d*-methamphetamine, benzoylecgonine, morphine and 11-nor-9-carboxy-THC using fortified urine samples. Similar observations among all of the different drug classes indicated that the interference by zinc is likely due to an alteration in antibody/antigen interaction or an inhibition of enzymatic activity. Our second finding that self-administration of zinc resulted in random urine total zinc concentrations ~5,000-fold less than the concentration found to interfere with EMIT-based testing indicates that increased urine zinc alone is an unlikely explanation for false negatives after zinc self-administration. Finally, we saw no effect on the EMIT results for any of the drugs fortified into urine collected after zinc self-administration, indicating the absence of secondary substances excreted and present at concentrations capable of interfering with EMIT-based testing as a result of zinc self-administration.

The observation that mg/mL concentrations of zinc interfere with EMIT-based testing is consistent with previous reports for ELISA-based testing in the production of false negatives (1); however, the considerably lower urine total zinc concentrations after self-administration reported in our study do not support the conclusion of zinc presence alone as the explanation for false-negative results. The proposed mechanism for the observed zinc interference with ELISA testing was an enhanced binding of

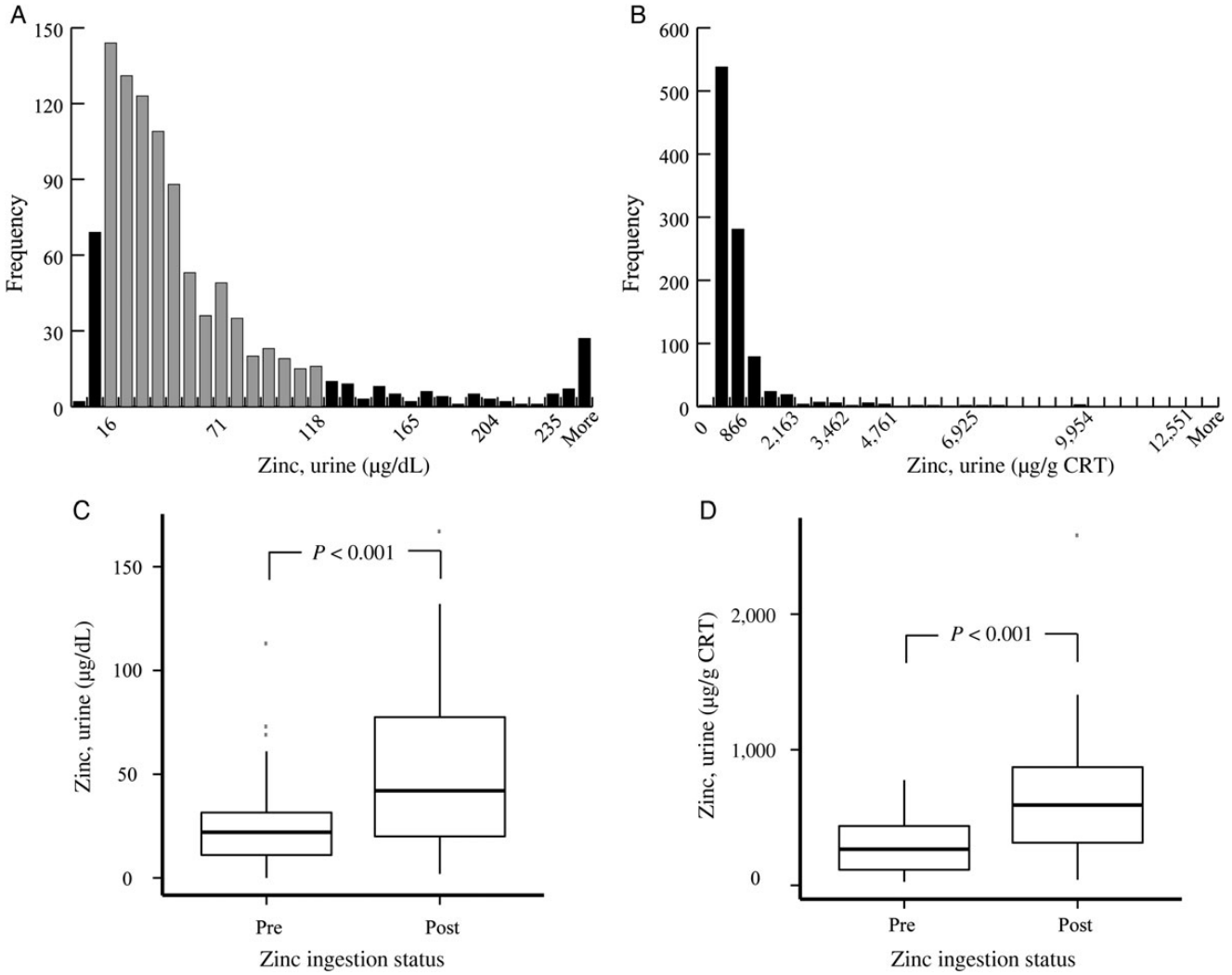

Figure 2. Distributions of urine total zinc concentrations. (A and B) Distribution of total zinc concentration in random urines (A) and creatinine normalized urines (B) from 1,031 samples submitted for routine clinical testing. Bins containing samples within the random urine reference interval of 15–120 µg/dL are shaded gray. (C and D) Boxplots of total zinc concentration in random urines (B) and random urines normalized to creatinine (C) collected before ('pre') and after ('post') zinc gluconate ingestion. Statistically significant increases were observed in the 'post' group for random and creatinine normalized concentrations (*P*-values <0.001).

Table I
Results of the zinc self-administration study, population characteristics and distributions of routine zinc concentrations

Age	Minimum	Maximum	Mean	
Male ($n = 14$)	24	59	42	
Female ($n = 17$)	23	64	47	
	Mean	Median	SD	Maximum
Zinc (µg/dL)				
Pre ($n = 31$)	27	19	221	113
Post ($n = 31$)	50	42	514	167
Difference (post − pre)	24	14	34	106
Routine urines	53	35	54	185[a]
Zinc (µg/g CRT)				
Pre ($n = 31$)	297	255	224	777
Post ($n = 31$)	666	563	512	2,581
Difference (post − pre)	369	269	393	1,881
Routine urines	683	394	1,104	2,038[a]

[a]95th percentile value.

drug conjugate, resulting in increased absorbance values and a negative result.

The false-negative results we observed for the EMIT-based testing are most likely due to the inhibition of the glucose-6-phosphate dehydrogenase enzyme (G-6-PD) by zinc as reported previously (4). Of note, the concentrations of zinc reported to inhibit G-6-PD were considerably less than required in our experiments (0.196 versus our 5 mg/mL minimum); however, those studies were conducted at pH 8.0, while the average urine pH in our study pre- and post-zinc ingestion were 6.51 and 6.45, respectively (optimum pH of G-6-PD is 7.6). Regardless, the concentration of zinc reported to inhibit G-6-PD activity is ~100 times higher than the maximum observed random total zinc concentration in our study (19,620 versus 167 µg/dL), further indicating that zinc presence alone is unlikely to cause false-negative results using EMIT-based testing.

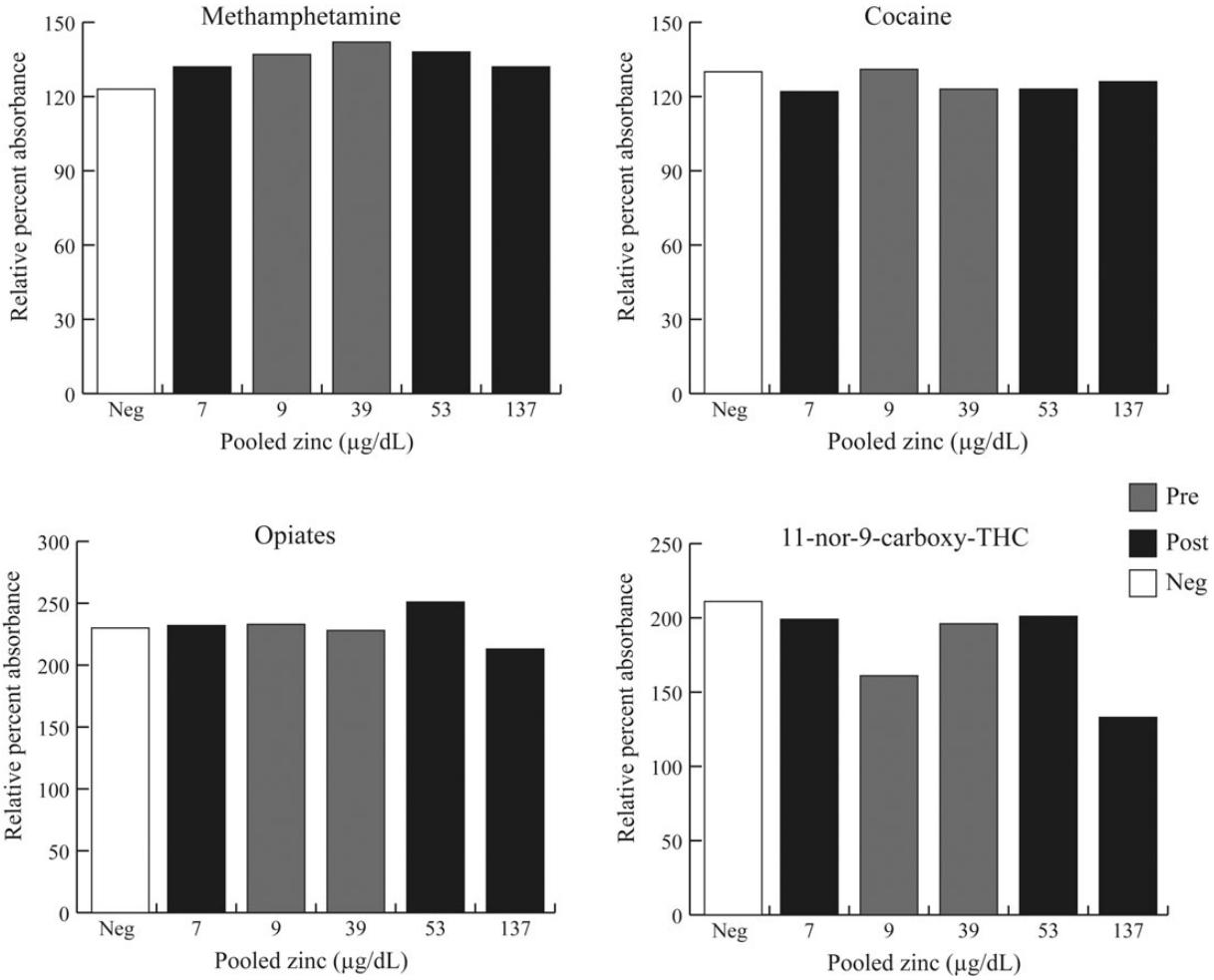

Figure 3. Relative absorbance for each drug class tested in pooled 'pre' and 'post' zinc ingestion samples. Negative urine (Neg, white bars), in addition to urine pools from 'pre' (gray bars) and 'post' (black bars) zinc ingestion samples were fortified at 400, 200, 800 and 40 ng/mL for *d*-methamphetamine, benzoylecgonine, morphine and 11-nor-9-carboxy-THC, respectively. All data were normalized to the absorbance of a prepared calibrator at the cutoff concentration for each assay (300 ng/mL of *d*-methamphetamine; 150 ng/mL of benzoylecgonine; 300 ng/mL of morphine and 20 ng/mL of 11-nor-9-carboxy-THC).

Conclusion

In summary, our results support the possibility that addition of zinc directly to urine can produce false-negative results with EMIT-based testing. In contrast, our results refute the possibility that self-administration of zinc can produce false-negative drug testing results due to elevated urine total zinc concentrations alone or the excretion of a secondary interfering substance. Of importance for the clinical laboratory, zinc added directly to urine in an attempt to circumvent a positive immunoassay-based drug-screen result is easily distinguishable from routine random urine total zinc concentrations.

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