Urinary Elimination of Cocaine Metabolites in Chronic Cocaine Users during Cessation

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Abstract

We previously showed that chronic cocaine use by active illicit users produced a longer plasma half-life than expected based on acute low-dose cocaine studies. Here we report urinary excretion patterns of cocaine metabolites as benzoylecgonine (BE) equivalents from 18 of the same individuals, housed for up to 14 days on a closed research unit. In addition, we evaluated whether creatinine normalization of BE equivalents increased mean detection time and reduced mean within-subject variability. All urine voids (N = 953) were individually assayed; BE equivalents were determined semi-quantitatively by FPIA. Compared to concentration in first void after admission, BE equivalents decreased to approximately 33%, 8%, and 4% at 24, 48, and 72 h, respectively. Mean ± SD (range) time to first negative specimen (BE equivalents < 300 ng/mL) was 43.6 ± 17.1 (16–66) h. BE equivalents fluctuated considerably across successive specimens; 69% of participants tested positive at least once after testing negative, and the mean time to last positive specimen was 57.5 ± 31.6 (11–147) h after the first specimen. Thus, mean cocaine metabolite detection times were consistent with prolonged elimination, with 63% of participants testing positive longer than the expected 48-h window of detection after admission to the unit. Mean time to last positive after last use of cocaine, known by self-report only, was approximately 81 ± 34 (34–162) h. Creatinine normalization, with the cut-off of 300 ng BE equivalents/mg creatinine, increased detection time: mean time to first negative specimen was 54.8 ± 20.7 (20–100) h, and mean time to last positive specimen was 88.4 ± 51.0 (35.6–235) h. Compared with the concentration in the first void after admission, BE equivalents/creatinine decreased to approximately 56%, 6%, and 5% at 24, 48, and 72 h. However, creatinine normalization did not reduce the fluctuation of BE equivalents across successive specimens. Thus, creatinine normalized values may be useful when the goal is to maximize the probability or duration of cocaine metabolite detection, but may be less useful in determining whether an individual has used cocaine since a previous specimen collection.

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Introduction

Urine testing for cocaine is practiced widely in workplaces, treatment programs, hospitals, and legal settings. In some situations, such as in the workplace, impaired-professionals programs, and drug courts, testing is a deterrent against drug use: testing positive for drugs results in a negative consequence, such as job loss or incarceration. In the context of treatment, testing is used for diagnosis of patients and monitoring their progress toward recovery; test results can affect treatment decisions. Clinical trials of substance-abuse therapies typically employ urine drug screens as a surrogate measure for cocaine use and as an outcome measure of therapeutic efficacy. Medications and other treatment interventions might be approved for use based on decreases in the percentages of urine specimens positive for cocaine metabolites (typically detected in screening immunoassays as benzoylecgonine [BE] equivalents). Given the key role of screening drug tests in a variety of settings, surprisingly few data have been published on results of testing urine specimens systematically collected from chronic, high-dose users of cocaine (referred to in the rest of this paper as "street" cocaine users).

Although the detection period for BE equivalents is generally reported to be about 48 h (1), there is evidence that the elimination of cocaine and its metabolites is protracted after long-term, high-dose cocaine use, resulting in a longer-than-expected detection period. A three-patient case report and a small-scale study, both using enzyme-multiplied immunoassay technique (EMIT), reported detection of BE equivalents in urine for more than 120 h (at a cut-off concentration of 300 ng/mL (2,3). Further, a highly sensitive and specific gas chromatography–mass spectrometry (GC–MS) assay (limit of detection 0.5 ng/mL) confirmed the presence of unmetabolized cocaine through the first 24 h of collection in saliva and for the first 4–5 days in urine of subjects who reported using 1–10 g (street quantities) per week for 1–10 years (4). In cocaine-using methadone-maintained outpatients providing single urine specimens thrice weekly, unmetabolized cocaine (> 25 ng/mL, GC–MS) was detected in 36.8% of all specimens and 62.7% of

all specimens positive for BE equivalents (> 300 ng/mL, EMIT) (5). This proportion of cocaine-positive to BE equivalents-positive specimens was higher than expected based on the 7- to 15-fold difference between the published plasma half-lives of cocaine (35 min to 1.5 h) and BE (7.5 h) (6–10).

The prolonged detection times seen in the urine of high-dose street users may reflect an alteration in cocaine pharmacokinetics. To investigate this possibility, our laboratory conducted a pharmacokinetic analysis of cocaine in plasma specimens collected over the first 12 h of cessation in street users (11). In these users, the terminal plasma half-lives of cocaine, BE, and ecgonine methyl ester were 3.8, 6.6, and 5.5 h, respectively. These half-lives of cocaine and ecgonine methyl ester were longer than those previously found following acute administration of cocaine, while the half-life of BE was similar to that found in earlier reports (6–8). Thus, regular use of cocaine may alter its disposition and elimination when compared to single or occasional use.

Although the plasma half-life of BE in street users did not differ from expected values, it remains unknown whether the same is true for the elimination rate of BE equivalents in urine. Any attempt to address this question should include a means of controlling for variations in urine concentration. In some cases, particularly when drug-positive urine specimens are linked to negative consequences, deliberate dilution of urine may occur. In fact, urine dilution is the main action of commercial products marketed for the purpose of defeating urine toxicology screens; these products are typically packaged with instructions encouraging the ingestion of large amounts of liquids (12). Unusually dilute urine specimens can be detected by measuring creatinine concentration and specific gravity. Guidelines recommended by the U.S. Department of Transportation for determining abnormally dilute urine include a measurement of creatinine concentration of less than 20 mg/dL and a specific gravity of less than 1.003 (13). Even when there has been no attempt at dilution, urine concentration will naturally vary with fluid consumption and excretion, introducing an unwanted source of variability in analyte concentrations. For example, successive urine specimens tested for the cannabinoid metabolite 11-nor-9-carboxy-Δ^9-tetrahydrocannabinol (THC-COOH) displayed a sawtooth-like pattern of variability; that pattern was smoothed by a creatinine-normalization procedure in which each specimen's THC-COOH concentration was divided by its creatinine concentration (14).

As a follow-up to our earlier evaluation of cocaine kinetics in the plasma of street users (11), we now report the urinary elimination patterns of BE equivalents in a subset of the same individuals. Specimens were tested by immunoassay for benzoylecgonine equivalents in a semi-quantitative mode; creatinine was measured by the Jaffé method (to be described in detail). In addition, we report on the effectiveness of creatinine normalization in extending the detection time of cocaine metabolites in urine and reducing within-subject variability. Collections were performed for up to 14 days, and all urine voids were collected and analyzed separately.

Methods

Participants

Eighteen individuals participated in the study; demographics and drug-use histories are summarized in Table I. To qualify, applicants had to report cocaine use on at least 14 days per month and for at least 1 year. Overall, participants reported substantial histories of cocaine use, with 7.3 ± 4.1 (mean ± SD) years' duration (range 1–17) and recent cocaine consumption of 1.0 ± 0.6 g/day (range 0.12–2.5), on 9.6 ± 3.2 days of the last 14 (range 1–14). All reported using cocaine by the smoked route. The self-reported time of last use prior to admission to the research unit was 19.8 ± 15.8 h (range 2.5–63).

Prior to participation, applicants underwent screening procedures including a complete physical and psychological examination. Only applicants with good venous access and no evidence of anemia or physical dependence on drugs or medications (except tobacco) were eligible to participate. This study was approved by the Institutional Review Board of the Intramural Research Program at the National Institute on Drug Abuse. Participants provided written informed consent and were paid for their participation.

Study procedures

Participants reported to the laboratory at approximately 8:30 a.m. on the day of admis-

Table I. Subject Characteristics and Self-Reported Drug Use

Subj.	Gender	Age	Weight (kg)	Self-Reported Cocaine Use: Grams/day of use	Time since last use (h)	Days used in last 14	Self-reported use of other drugs*
B	M	30	68.1	1.3	2.5	13	e, m, f, p
C	M	36	83.5	0.5	36	11	e, m, h
D	M	30	68.3	1.0	15	8	e
F	F	42	59.0	1.0	NR	13	e, m, h, t, f, p
G	F	40	68.6	0.5	13	10	e, m, h, f
H	F	41	47.2	0.5	13	8	e, h, m, f, a
I	M	39	101.7	0.8	NR	14	e, h, m, t, f
J	M	31	89.0	2.0	15	12	e, h, m
K	M	33	55.8	0.1	NR	10	e, h, m
L	F	39	52.2	1.0	39	9	e, h, m, t, f
M	F	38	56.8	0.5	15	12	e, m
N	F	38	90.8	1.3	6	11	e, h, m
O	M	37	63.6	1.0	15	12	e, h, m, t
P	M	38	70.1	1.0	15	10	e, h, m
Q	M	31	69.0	1.0	NR	10	e, h, o, m, t, a, b, f
R	M	39	69.0	2.0	15	10	e, m, o, f
S	F	40	105.3	2.5	15	13	e, m, a, f, p, i, d
T	M	39	71.7	0.5	63	7	e, h, m

* Abbreviations: a, amphetamines; b, barbiturates; d, antidepressants; e, alcohol; f, hallucinogens; h, heroin; i, inhalants; m, marijuana; o, opiates; p, PCP; t, tranquilizers; M, male; F, female; and NR, not reported.

sion. On the first day of the study, they participated in a 12-h experimental session after which they were housed on the closed inpatient ward for up to 14 days. No drugs or medications were administered during the study. Meals, non-caffeinated beverages, and water were freely available, and recreational activities (television, reading material, and games) were provided. All urine voids were collected throughout the participants' stay. Each void was refrigerated, and time and volume were recorded. At the end of each day, aliquots of individual voids were transferred to smaller containers and frozen at –20°C until analysis.

Specimen analysis

All urine specimens were analyzed semi-quantitatively for cocaine metabolite by fluorescence polarization immunoassay (FPIA) on freshly thawed aliquots. FPIA was performed with TDx Cocaine Metabolite Assay reagents on a TDx instrument (Abbott Laboratories, Abbott Park, IL) in accordance with the manufacturer's recommended procedures. According to the product's package insert, the cross-reactivity of this assay for benzoylecgonine was 100% and < 1% for cocaine, ecgonine, and ecgonine methyl ester (15). As part of an earlier study evaluating the presence of cocaine and its metabolites in neonatal meconium, our group found that the assay had less than a 1% cross-reactivity for ecgonine ethyl ester, cocaethylene, norcocaine, norcocaethylene, benzoylnorecgonine, *m*-hydroxycocaine, and *p*-hydroxycocaine, and a cross-reactivity of 4.1% for cocaine itself (16). However, two compounds closely related to BE (*m*-hydroxybenzoylecgonine and *p*-hydroxybenzoylecgonine) demonstrated cross-reactivities of 95% and 108%, respectively (16). Because of the potential contributions of these metabolites to the immunoassay results, we describe our findings with the term "BE equivalents". The assay has been shown not to cross-react with many other commonly prescribed or abused drugs (15). The limit of detection of the immunoassay was 30 ng/mL with a linear range of 30 to 5000 ng/mL. Specimens that contained BE equivalents greater than 5000 ng/mL were diluted with reagent buffer until concentrations fell within the linear range of the assay. Creatinine concentrations were determined by the Jaffé method with Boehringer Mannheim Diagnostic reagents on a Hitachi 704 analyzer (Boehringer Mannheim, Indianapolis, IN).

Data analysis

Distributions of values of BE equivalents (ng/mL), creatinine (mg/dL), and BE equivalents/creatinine (ng BE equivalents/mg creatinine) were examined descriptively with the Univariate procedure in SAS V. 8.0. The first urine specimen obtained was identified as the "intake" specimen, and the time of its collection was defined as time 0.

Concentrations at 24, 48, and 72 h after intake were determined from the specimen collected closest to the time of interest, whether before or after. Of the 50 specimens used to

Table II. Individual and Mean BE* Equivalents and Times of Positive and Negative Specimens at a BE Equivalents Cut-off of 300 ng/mL

	BE Equivalents				Time (h) to	Highest BE Equivalents			Time of Last	Time to First Negative	Num. Pos. After First Negative	Time of Last Positive
Subject	Intake	24 h†	48 h†	72 h†	highest	0–24 h	24–48 h	48–72 h	Specimen	Specimen	Specimen	Specimen
B	277,709	15,441	1389	406	0.0	277,709	15,441	1389	239	54.5	2	72.6
C	25,232	2704	655	152	0.0	25,232	2704	655	191	59.5	0	55.8
D	14,777	7,697	872	44	20.6‡	15,361	2360	872	152	54.2	0	50.8
F	680	219	190	62	5.3	1486	339	186	207	21.3	4	41.1
G	20,649	1972	803	376	0.0	20,649	1973	508	321	75.5	2	93.8
H	20,189	801	186	465	0.0	20,189	939	465	141	44.6	1	71.2
I	17,603	546	99	129	0.0	17,603	749	203	97	30.6	1	35.6
J§	719	1426	–	–	6.4	13,308	1426	–	45	–	–	–
K	17,538	1752	362	23	0.0	17,538	1576	158	236	65.9	0	58.2
L	3083	513	241	303	2.0	5893	1003	449	236	49.2	4	71.6
M	166,424	4743	286	87	0.0	166,424	4743	492	140	46.3	3	54.5
N	2164	5516	437	178	8.6	68,575	2813	522	130	44.7	4	55.6
O	3406	862	338	104	0.0	3406	1150	338	119	31.3	4	49.0
P	5297	165	113	73	3.6	9763	191	164	95	20.4	0	18.9
Q	630	249	167	89	3.0	1126	249	167	92	16.0	0	11.1
R	4992	2723	570	231	11.2	7432	1521	570	160	52.9	5	146.8
S	14,679	1731	184	83	0.0	14,679	1730	147	160	31.2	1	33.8
T§	1700	413	–	–	0.0	1700	–	–	24	–	–	–
Mean	33,191	2977	431	176	2.9	38,211	2464	455	161	43.6	1.9	57.5
SD	75,406	3963	351	139	5.4	71,189	3651	327	71	17.1	1.8	31.6

* BE = benzoylecgonine. Units of BE equivalents are ng/mL; time is in hours relative to the intake specimen (time 0.0 h).
† Concentration in specimen collected closest to (either before or after) 24, 48, or 72 h after intake specimen.
‡ No specimens were collected between time 0 and 20.6 h.
§ Stayed less than 48 h; urine BE equivalents did not reach < 300 ng/mL; not included in means because data were not available for all variables.

make these determinations, 37 (74%) were collected within 1 h of the designated time, and 11 more (22%) were collected within 2 h of the designated time. Two specimens (the 24-h specimens from participants G and B, respectively) were collected 2.2 h and 5.5 h after the designated time.

Values of BE equivalents and BE equivalents/creatinine were classified as positive or negative at a range of possible cut-off values from 500 to 100 (the units for BE equivalents were ng/mL; the units for BE equivalents/creatinine were ng BE equivalents/mg creatinine). Concentrations were graphed to determine a cut-off for creatinine-normalized BE equivalents that would give detection rates comparable with those obtained with the standard 300-ng/mL cut-off for non-normalized BE equivalents. To determine whether creatinine normalization changed the degree of variability in BE equivalents, coefficients of variation (CVs) were calculated for each participant's normalized and non-normalized BE equivalents, and these two sets were compared with a one-sample t-test.

Results

A total of 953 urine specimens were collected (ad libitum) from 18 chronic cocaine users over residence periods ranging from 24 to 321 h (Table II). Patterns of urine BE equivalents and BE equivalents/creatinine concentrations are shown for each participant for up to 144 h in Figure 1. All 18 participants tested positive for cocaine metabolites (BE equivalents ≥ 300 ng/mL) after admission onto the research unit.

Benzoylecgonine concentrations

Urinary BE equivalents ranged from 630 to 277,709 ng/mL at time 0 (Table II). By 24, 48, and 72 h after intake, mean BE equivalents had decreased to 33.5%, 7.7%, and 3.6% of the initial concentration. At 48 h (or the closest void to 48 h), urine collected from 8 out of 16 participants (50%) still on the unit was positive for BE equivalents (≥ 300 ng/mL). At 72 h, four (25%) participants gave positive specimens; however, only two of these participants had also been positive at 48 h; this observation illustrates the fluctuation of BE equivalents around the 300-ng/mL cut-off.

For 10 of the 18 participants (56%), the highest concentration of BE equivalents was detected in the first urine specimen collected (Table II). In seven of the other participants, the mean time of highest concentration was 5.8 ± 3.3 h (range 2–11.2) after the first specimen collected. (For the remaining participant, D, the highest concentration of BE equivalents occurred 20.6 h after intake; however, no specimen was collected prior to this time.) Although there was substantial fluctuation over time, the highest concentration of BE equivalents in each of the

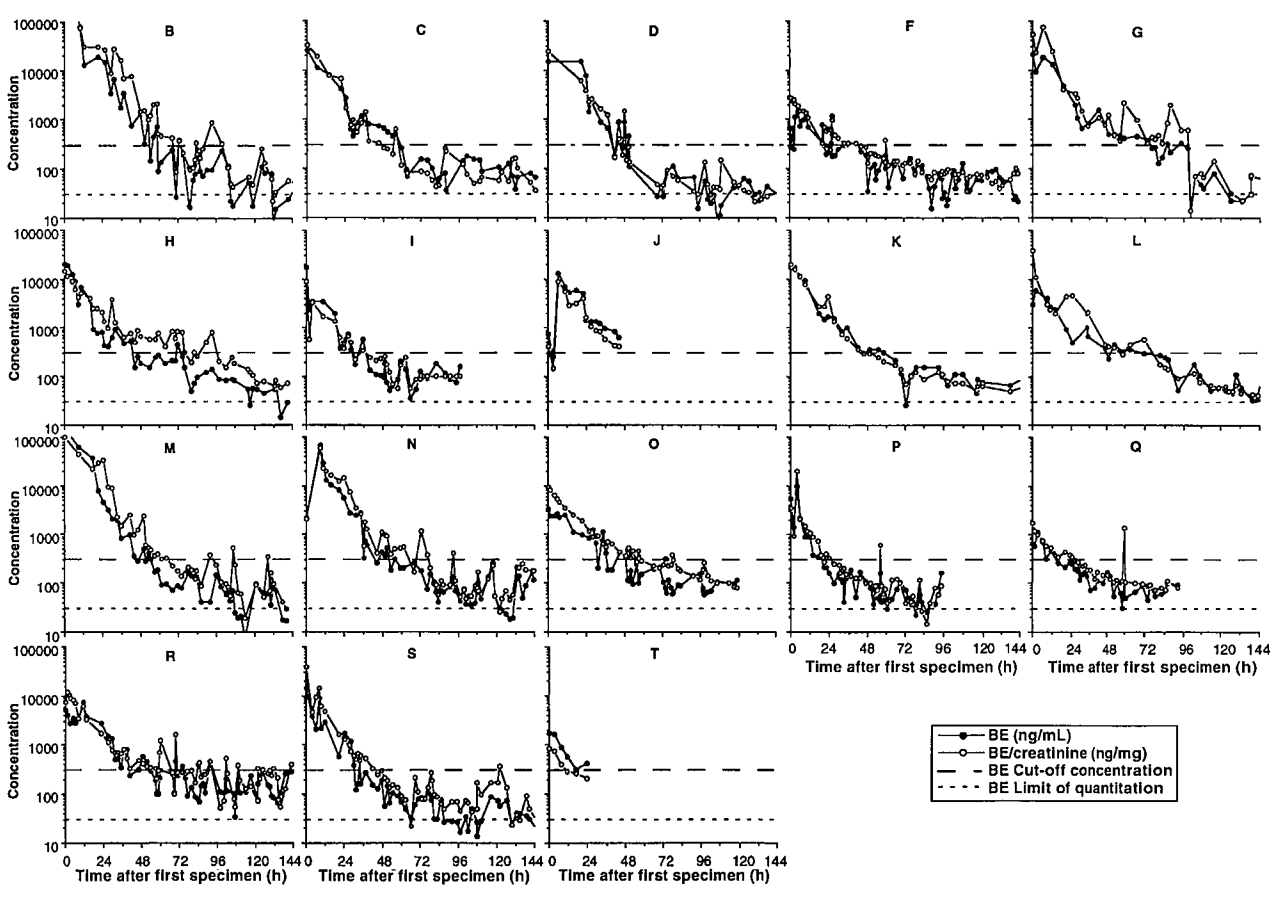

Figure 1. Benzoylecgonine equivalents (solid circles) and normalized BE equivalents (ng BE equivalents/mg creatinine, open circles) determined by FPIA. Results are semi-quantitative concentrations of sequential urine specimens of individual participants. Dashed lines indicate the 300-ng/mL concentration cut-off, and dotted lines indicate the 30-ng/mL limit of quantitation for BE equivalents.

24-h intervals after intake, 0–24, 24–48, and 48–72 h, declined consistently. The highest concentration of BE equivalents was greater than 300 ng/mL in 15 of 17 participants (88%) between 24 and 48 h and in 10 of 16 participants (63%) between 48 to 72 h after intake.

Sixteen of 18 participants remained on the residential unit long enough to test negative (BE equivalents < 300 ng/mL) (Table II). In these 16 participants, the mean (range) time to first negative test was 43.6 ± 17.1 (16.0–75.5) h. Eleven of these 16 participants (69%) tested positive at least once after testing negative. Due to fluctuations in BE equivalents, the mean time to the last positive test was 57.5 ± 31.6 (11.1–146.8) h—a mean of 13.5 ± 22.1 h (median 7 h, range 0–94 h) longer than the time to first negative test.

BE equivalents/creatinine concentrations

The mean urine creatinine concentration in individual participants was 88.8 ± 39.1 mg/dL (range 49–193). BE equivalents/creatinine concentrations, along with nonnormalized concentrations, are shown in Figure 1. Visual inspection of the data suggested that creatinine normalization smoothed the concentration time curve for some participants (such as Participant O) while possibly having the opposite effect for others (such as Participant M). Overall, however, creatinine normalization had no significant effect on the CV, which was 253.9% ± 100.0 (range 66.2%–420.3%) for non-normalized concentrations and 263.5% ± 107.9 (range 59.0%–405.2%) for normalized concentrations, $t(17) = 0.69$, $p = .49$.

The mean concentration of BE equivalents/creatinine at intake was 43,373 ± 81,837 ng/mg (range 418–334,590) (Table III). By 24, 48, and 72 h after intake, the mean concentration had decreased to 56.1%, 6.5% and 5.0% of its initial value. No recommended cut-off has been established for creatinine-normalized BE equivalents; for ease of comparison with non-normalized BE equivalents, we chose a cut-off of 300 ng/mg. Using this cut-off, we found that 9 of the 16 participants (56%) still on the unit gave positive urine specimens at 48 h. Five (31%) were positive at 72 h; all five had also been positive at 48 h. The highest BE equivalents/creatinine occurred at intake for 13 of the 18 participants (72%), including Participant D, whose non-normalized concentration of BE equivalents was highest at 20.8 h. As with non-normalized BE equivalents, the highest BE equivalents/creatinine concentrations declined across each of the first three 24-h intervals after intake.

The primary impact of creatinine normalization was to increase detection time for BE equivalents (Table III; Figure 2). Between 24 and 48 h, 16 of 17 participants had at least one positive specimen (≥ 300 ng/mg); between 48 to 72 h, 15 of 16 participants had at least one positive specimen. Mean ± SD time to first negative was 54.8 ± 22.7 h. However, creatinine normalization did not reduce variability around the cut-off concentration. Using non-normalized BE equivalents, the mean number of positives after the first negative was 1.9 ± 1.8; with creatinine normalization, it was 2.8 ± 3.3. These means were

Table III. Individual and Mean Creatinine-Normalized BE* Equivalents and Times of Positive and Negative Specimens at a Cut-off of 300 ng BE Equivalents/mg Creatinine

	BE Equivalents/Creatinine					Highest BE Equivalents/Creatinine						
Subject	Intake	24 h†	48 h†	72 h†	Time (h) to Peak	0–24 h	24–48 h	48–72 h	Time of Last Specimen	Time to First Negative Specimen	Num. Pos. After First Negative Specimen	Time of Last Pos. Specimen
B	334,590	27,090	1494	386	0.0	334,590	28,574	2092	239	70.7	4	99.3
C	32,769	1722	258	87	0.0	32,769	1412	654	191	48.1	1	55.8
D	23,833	3792	145	41	0.0	23,833	2620	1504	152	50.8	0	48.3
F	2721	625	276	115	0.0	2721	625	372	207	44.5	1	49.3
G	54,339	3343	732	324	6.5	54,339	3343	2159	321	100.4	2	234.8
H	14,737	2107	664	682	0.0	14,737	3766	862	141	79.9	3	93.6
I	8890	348	240	89	0.0	8890	675	251	97	30.6	1	35.6
J‡	418	1677	–	–	6.4	9054	1677	–	45	–	–	–
K	19,929	4494	299	71	0.0	19,929	1358	299	236	45.5	0	41.8
L	38,534	4660	416	572	0.0	38,534	4660	572	236	59.1	2	71.6
M	100,863	33,881	1244	178	0.0	100,863	33,881	2347	140	72.9	3	128.2
N	2121	14,517	1119	1188	8.6	60,686	7294	1188	130	68.4	3	92.5
O	9462	1165	368	232	0.0	9462	1164	540	119	55.8	5	79.2
P	3462	330	101	39	3.7	19,925	245	610	95	20.4	3	56.6
Q	1750	346	150	98	0.0	1750	376	1352	92	26.5	1	59.9
R	7341	1691	416	272	2.8	12,167	1110	1676	160	57.8	14	146.8
S	38,628	1282	307	81	0.0	38,628	1282	278	160	45.3	2	122.0
T‡	837	206	–	–	0.0	837	–	–	24	11.1	–	–
Mean	43,373	6339	603	285	1.5	38,211	5533	1047	161	54.8	2.8	88.4
SD	81,837	10,103	479	315	2.8	71,189	9884	723	71	22.7	3.3	51.0

* BE = benzoylecgonine. Units of creatinine-normalized BE equivalents are ng BE equivalents/mg creatinine; time is in hours relative to the intake specimen (time 0.0 h).
† Concentration in specimen collected closest to (either before or after) 24, 48, or 72 h after intake specimen.
‡ Not included in the mean because data were not available for all times.

not significantly different in a paired t-test, $t(15) = 1.33, p = .20$. Mean time to last positive test was 88.4 ± 51.0 h—a mean of 30 ± 37.0 h (median 17 h, range 0–134 h) longer than the time to first negative test.

Figure 2 shows the effect of creatinine normalization of BE equivalents on the time to reach various concentration cut-offs and on the proportion of specimens above each cut-off. As one would have expected, both time to first negative test and proportion of positive tests increased as the concentration cut-off was lowered. Values for both variables were also consistently higher for creatinine-normalized BE equivalents than for non-normalized values at each of the cut-off concentrations. The time to first negative test (approximately 44 h) at a cut-off of 450 ng/mg was approximately equal to that of non-normalized BE equivalents at the 300-ng/mL cut-off.

Discussion

This paper presents urine test results for BE equivalents from 18 chronic, high-dose cocaine users living on a closed research ward. All urine voids were collected individually and stored for later analyses. Specimens were analyzed by semi-quantitative immunoassay (FPIA), a typical analytic method available in many treatment settings. The present study addresses two main issues: duration of detection of BE equivalents in the urine of chronic, high-dose cocaine users and the impact of creatinine normalization of BE equivalents on detection of cocaine use.

The detection times of cocaine metabolites in these self-identified chronic cocaine users was relatively long. Although the first negative specimens occurred an average of 43.6 h after intake onto the residential unit, the last positive specimens were detected an average of 57.5 h after intake. The last cocaine use by self-report was on average about 20 h prior to intake. Mean cocaine metabolite detection times were, thus, somewhat longer than the expected 48-h window of detection for acute cocaine use (1). This long detection time is consistent with the prolonged cocaine plasma elimination times that we detected among the same cocaine users in our previous report (11). It is also consistent with results from the prior published study most closely comparable to ours (17), in which urine cocaine metabolite was monitored by FPIA for up to 90 days on an inpatient ward in 11 participants (8 of whom were still above 300 ng/mL at study intake). Participants in that study remained positive for a mean of 4.8 days (range 3–6 days), with day 0 defined not as the day of study intake, but as the day of their last self-reported use prior to intake. Analyzing our data the same way, we found that the last positive occurred an average of 3.4 days (range 1.4–6.7 days) after the last self-reported use. Given the limitations of retrospective self-reports of last use, the two findings are roughly in accord.

We found substantial fluctuation in BE equivalents from specimen to specimen, such that at the 300-ng/mL cut-off, participants intermittently tested positive after having tested negative, as shown in Figure 1 and Table II. On average, the last positive specimen occurred 13.5 h after the first negative specimen; for three participants (L, H, and R), positive specimens

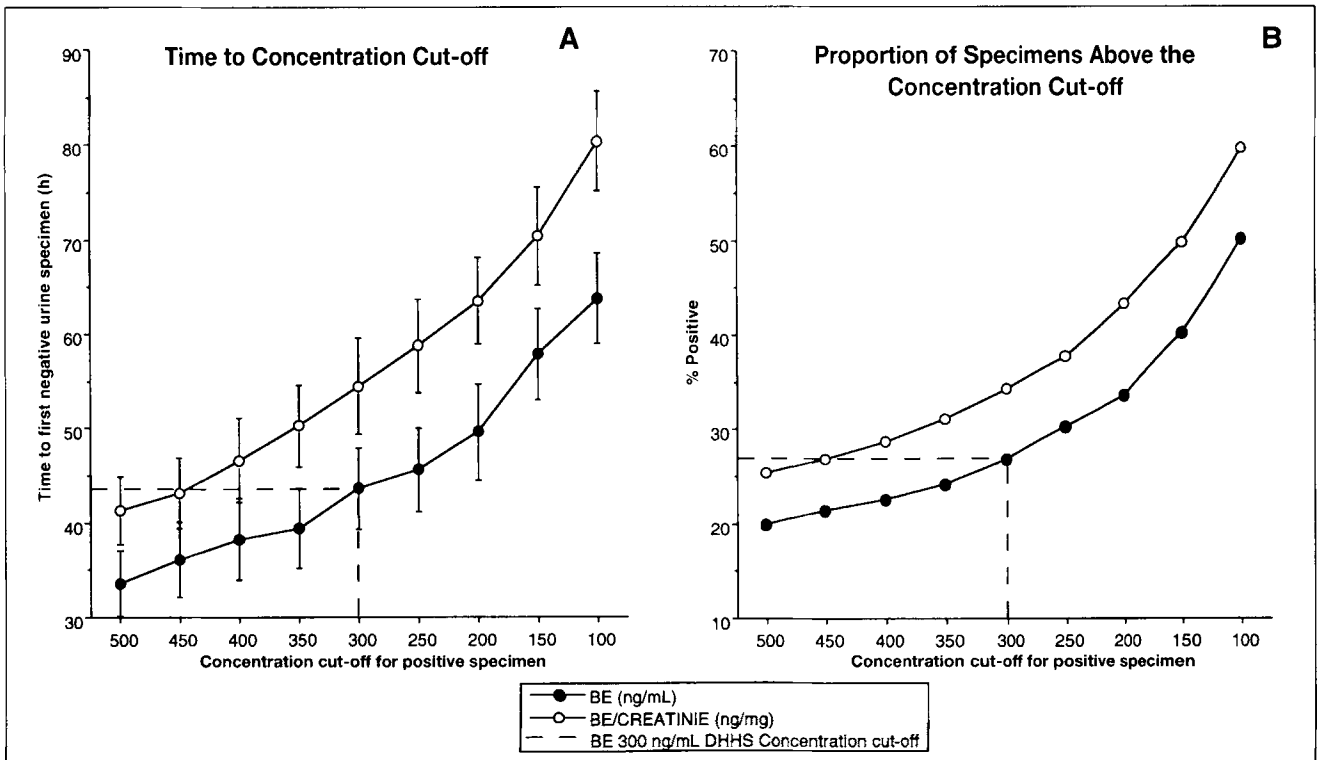

Figure 2. Effects of creatinine normalization on results for urine benzoylecgonine equivalents in terms of (A) time to first negative urine specimen and (B) percentage of specimens positive, at cut-off concentrations of 100 to 500 ng/mL BE equivalents (nonnormalized; open circles) and 100 to 500 ng BE equivalents/mg creatinine (normalized; solid circles). Brackets in (A) indicate standard errors; dashed vertical and horizontal lines in (A) and (B) indicate results at a positive/negative cut-off of 300 ng/mL for non-normalized BE equivalents.

occurred 22, 27, and 94 h after the first negative specimen. The implication of this finding is that, on repeated testing, individuals could appear to test "newly" positive for cocaine without actually having used cocaine since the previous specimen collection. The degree of fluctuation seen here was not seen by Peters et al. (17); however, in that study, each participant's FPIA results were pooled across each 24-h collection period, greatly reducing variability.

Our second main finding was that, at any given cut-off, creatinine normalization can increase the proportion of specimens positive for cocaine metabolites and increase detection time (Figure 2). To our knowledge, there is no suggested concentration cut-off for creatinine-normalized BE equivalents. These and similar data may be useful for selecting an appropriate cut-off. The purpose of using creatinine normalization will be important in choosing the cut-off. A low cut-off might be applied if the purpose were to increase the likelihood and duration of detection when large amounts of fluid are consumed. However, if the purpose is to maintain the approximate proportion of screens that test positive, a higher cut-off concentration, such as 450 ng/mg, appears to be appropriate.

Creatinine normalization did not systematically smooth the urinary elimination curve (Figure 1 and Table III); in fact, the number of positive specimens detected after the first negative specimen tended to increase, using the 300-ng/mL cut-off (Tables II and III), though this effect did not reach statistical significance. Furthermore, the mean time to last positive was 88.4 h, and the mean time to first negative test was 54.8 h. Thus, the mean difference between first negative and last positive was more than 30 h for the creatinine-normalized values, approximately twice as long as for nonnormalized BE equivalents (time to last positive 57.5 h vs. time to first negative 43.6 h, a difference of 13.9 h). For one participant (G), a positive specimen (with BE equivalents and creatinine values of 45.79 ng/mL and 29 mg/dL, respectively) occurred fully 134 h after the first negative specimen. This was at an arbitrary cut-off of 300 ng BE equivalents/mg creatinine, but our inspection of the data suggests that similar fluctuation would occur at higher cut-off points as well.

The failure of creatinine normalization to smooth the cocaine metabolite elimination curve in most participants contrasts with earlier findings from our laboratory in which creatinine normalization did smooth the elimination curve for the cannabinoid metabolite THC-COOH (14). We can only speculate on the reasons for such discrepant findings. In a review of the potential pitfalls of creatinine normalization, Boeniger et al. (18) suggests that creatinine normalization is most effective for substances whose renal excretion rate, like that of creatinine, does not appreciably vary with urine flow rate. Various processes occurring in the nephron (such as active secretion into the tubules, or passive reabsorption from them) could make an analyte's renal-elimination profile differ from that of creatinine. To our knowledge, there has been little systematic study of how these processes may differentially affect renal elimination of THC-COOH or cocaine metabolite.

Our finding that creatinine normalization increased detection time for cocaine metabolite departs from the findings of Peters et al. (17). Using a cut-off of 300 ng BE equivalents/mg creatinine, the authors found no net effect: detection time increased by one day in one participant, decreased one day in another participant, and was unchanged in the other six (17). However, this analysis was based on specimens pooled across 24-h collection periods, which may have reduced the impact of brief spikes in urinary BE equivalents due to alterations in hydration state. Such spikes in BE equivalents can be seen in the present study, and close inspection of the figures in Peters et al. (17) suggests that some participants in their study showed similar spikes, intermittently testing positive and negative. Peters et al. (17) did not comment on that finding but did cite its occurrence in two earlier studies (2,3). Their recommendation for clinical researchers attempting to interpret data from outpatient specimens (typically collected thrice weekly or less often) was to examine successive values of quantitative BE equivalents, and only interpret "substantial" or "significant" increases as indicators of new use. We have published New Use criteria to help make this determination in thrice-weekly specimens (19). When the goal is to detect new use rather than to maximize cocaine metabolite detection time, the New Use criteria appear preferable to any single creatinine-normalized cut-off. These criteria were based on established pharmacokinetic parameters of cocaine and BE and included urine creatinine measures for the normalization of variations in water intake and excretion. The algorithm was explicitly designed to distinguish specimens that were positive due to recent use of cocaine (since last urine collection) from those specimens that were positive due to carryover from earlier use. Concentrations of BE equivalents (determined in semi-quantitative immunoassay) in newly collected urine specimens were compared with those of specimens collected 24 to 72 h earlier.

To evaluate the generalizability of the present results, the impact of creatinine normalization on detection of cocaine use needs to be tested in other populations and other settings. Boeniger et al. (18) described increases in urinary creatinine concentrations with lean body mass, high-meat diet, pregnancy, and daytime versus nighttime specimen collection, and higher concentrations in males than in females, and decreases with age (in adults), along with a biphasic pattern of decrease and increase after exercise. Any of these factors could shorten or lengthen the detection time for creatinine-normalized BE equivalents, a possibility that should be borne in mind, especially when a positive test is accompanied by adverse consequences.

Limitations of the present study include the collection of only minimal cocaine use parameters; times and amounts of last cocaine use were known only by self-report. We were able to calculate the minimum duration of drug-use detection, but not the maximum duration. The fact that participants were living on a secure residential unit, with freely available food and water and limited room for physical activities, could have had an impact on the excretion of cocaine metabolite. However, the controlled environment enabled us to collect all individual urine specimens and to ensure that additional drug use did not occur. Few such data are available due to the cost of conducting clinical studies and of analyzing large numbers of individual specimens. Finally, we used semi-quantitative immunoassay, with no confirmation by GC–MS. The value of this approach lies in

the fact that nearly all drug screening programs initially test specimens by immunoassay, and that semi-quantitative immunoassays are more likely than GC–MS to be within the financial and logistical reach of treatment programs. The data from this study could serve to improve the interpretation of qualitative or semi-quantitative urine drug tests for cocaine in clinical practice.

Overall, the present study showed that cocaine use can be detected in chronic, high-dose users for a prolonged period of time, that fluctuation of negative and positive urine tests around the cut-off must be expected, and that this fluctuation may not be reduced by creatinine normalization, although creatinine normalization can increase cocaine metabolite detection times.

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