Lack of Association Between Urinary Creatinine and Ethanol Concentrations and Urine/Blood Ratio of Ethanol in Two Successive Voids from Drinking Drivers

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Abstract

The relationship between urinary ethanol concentration, urine/blood ratio of ethanol, and urinary creatinine content was investigated by the analysis of two successive voids from 40 individuals apprehended for driving under the influence of alcohol (DUI) in Sweden. The first specimen of urine was collected 24 ± 17 min (mean plus or minus standard deviation) before sampling blood, and the second specimen was collected 46 ± 30 min after blood sampling. The mean blood-alcohol concentration (BAC) was 2.21 ± 0.76 g/L, and the corresponding concentrations in the urine (UAC) were 2.88 ± 1.08 g/L for the first void and 2.68 ± 1.01 g/L for the second void. The mean urine/blood ratios of ethanol were 1.31 ± 0.21 for the first void and 1.20 ± 0.11 for the second void; the difference of 0.11 ± 0.24 was statistically significant (t = 3.08, p < 0.01). The concentrations of creatinine in urine from DUI suspects were 0.72 ± 0.64 g/L for the first void and 0.86 ± 0.73 g/L for the second void; there was no significant difference (t = 1.45, p > 0.05). The urinary creatinine content in specimens from drunk drivers was significantly less than the first morning voids from 3313 prison inmates (1.78 ± 0.74 g/L). No significant correlations existed between UAC and urinary creatinine content (r = -0.14) between urine/blood ratios of ethanol and urinary creatinine (r = -0.19). However, the concentrations of ethanol in blood and urine were highly correlated; they were r = 0.94 ± 0.055 (p < 0.001) for first void and r = 0.96 ± 0.045 (p < 0.001) for the second void. This study demonstrates that the relative dilution of the urine specimens, as reflected in creatinine content, is not associated with the concentration of ethanol in the urine samples or with the UAC/BAC ratio.

Introduction

Measuring urinary creatinine is often included in drugs of abuse screening programs to monitor and control for highly dilute specimens. The concentration of creatinine in urine decrease sharply after drinking water, alcoholic beverages, or taking diuretic drugs (1–3). After increasing the production of urine (e.g., by drinking water), the concentrations of drugs of abuse such as cannabis and its metabolites decrease, making it more likely to obtain results below a certain critical threshold (4,5). Some investigators have suggested that the results of urinary drug testing should be reported per milligram of creatinine instead of per liter of urine whenever unusually dilute specimens are encountered as reflected in the urinary creatinine content (3,6). The analysis of urinary creatinine also provides a check on whether the urine specimens might have been diluted in vitro, that is, after voiding (7). Attempts to adulterate urine specimens in various ways such as by dilution with water or other liquids are not uncommon, especially among drug addicts and prison inmates undergoing rehabilitation (7–9).

Most analytical systems currently being used for drugs-of-abuse screening in urine make use of various immunoassay technologies, and these procedures can easily be modified to include the determination of urinary ethanol (10–12). There seems to be a growing interest in measuring and reporting the concentration of ethanol in urine along with the concentrations of illicit drugs of abuse (13,14). This raises the question of whether water-induced or alcohol-induced diuresis, as reflected in the concentration of creatinine and the relative dilution of specimens, needs to be considered when the urinary alcohol results are interpreted by medical review officers.

Two successive urinary voids were collected from suspected drinking drivers to study the impact of dilution of the urine specimens as reflected in creatinine content on the concentration of ethanol and the urine/blood ratios of ethanol. Urine was voided once before and once after a specimen of venous blood was obtained from each suspected drinking driver. The blood-alcohol (BAC) and urine-alcohol (UAC) concentrations and UAC/BAC ratios were determined and compared with the concentration of creatinine in the urine specimens.

Materials and Methods

Subjects

Specimens of blood and urine were obtained from 40 men apprehended in Sweden for driving under the influence of...
alcohol. Because many DUI suspects are not arrested while at the wheel, they have the opportunity to claim consumption of alcohol after driving and before providing body fluids for forensic analysis of alcohol. When this happens, the police must be prepared to obtain two urinary voids in addition to a sample of venous blood. The first specimen of urine should be taken as soon as possible after making the arrest, and the second specimen should be taken approximately 1 h later and close to the time of venous blood sampling. The concentration ratios of alcohol in blood and urine and the magnitude and direction of change in UAC between the two voids provides useful information to establish if recent consumption of alcohol has occurred. In this way, the allegation of drinking after the offence can be confirmed or challenged (15).

Accordingly, there are long traditions in Sweden for analyzing and interpreting the concentrations of alcohol in blood and urine samples in traffic law enforcement (16). It should be noted that DUI suspects cannot be prosecuted in Sweden on the basis of the UAC alone. The material for this study included 40 cases of DUI selected from those sent to the National Laboratory of Forensic Toxicology (Linköping, Sweden); both venous blood and two successive urinary voids were available for all cases.

Collection of the specimens of blood and urine

Blood samples were collected in evacuated tubes (Ivers-Lee Division of Becton Dickinson, West Caldwell, NJ) containing 100 mg sodium fluoride and 25 mg potassium oxalate as preservatives. Two tubes of blood were filled in rapid succession, and aliquots for analysis of ethanol were removed from both of these tubes. The subjects were observed during the collection of urine to ensure that the specimens were not adulterated. The total volume voided was recorded, and approximately 10 mL was transferred into a plastic screw-capped tube that contained a tablet of sodium fluoride (100 mg) as preservative.

Urine specimens (N = 3313) were also collected from prison inmates who were mostly healthy individuals not exposed to alcohol and not abusing drugs, and this gives a reference range for urinary creatinine. The first morning void was also collected from one healthy volunteer for 39 consecutive days.

Determination of urinary ethanol and creatinine content

The concentrations of ethanol in blood and urine were determined by headspace gas chromatography (GC) as described in detail elsewhere (17). All determinations of ethanol were made in duplicate (urine) or triplicate (blood), and the mean result was reported to two decimal places as grams ethanol per liter (g/L). The coefficient of variation of the GC method was 0.8% at a mean concentration of 1.0 g/L. Urinary creatinine was determined by the Jaffe method with reagents purchased from Boehringer-Mannheim and run on a Hitachi 717 analyzer. A single determination of creatinine was made on each specimen, and the within-run coefficient of variation (CV) of the method was 2% at a mean concentration of creatinine of 1.0 g/L. The UAC in the first and second voids were used to estimate the person’s BAC by dividing by an assumed UAC/BAC ratio of 1.33:1 (18,19).
Results

Figure 1A shows a histogram of urinary creatinine in 3313 specimens from prison inmates, and these results provide a reference range. The mean concentration of urinary creatinine was 1.78 g/L (median 1.70), and the distribution was somewhat skewed with a coefficient of variation of 41%, a coefficient of skewness of 0.651, and a coefficient of kurtosis of 0.486. Figure 1B shows a frequency distribution of the concentrations of creatinine in 39 consecutive first morning voids from one healthy male subject. Here the mean was $1.73 \pm 0.433$ g/L (± SD), which was not significantly different from the mean value for prison inmates ($p > 0.05$). However, the creatinine content of the urine specimens from DUI suspects (Table I) was significantly less than for the prison inmates and the healthy volunteer ($p < 0.001$). The change in urinary creatinine between the first and second voids was not statistically significant ($t = 1.45, p > 0.05$).

Table I presents mean values and variability for the concentrations of ethanol in urine, the urine/blood ratios of ethanol, and the concentration of creatinine in both first and second voids from 40 drinking drivers. The mean BAC was $2.20 \pm 0.76$ g/L, which was in good agreement with the estimated BAC ($2.22 \pm 0.83$ g/L) for the first void (UAC/1.33). The mean difference in BAC (observed – estimated) was $-0.02 \pm 0.047$ (± SE), which was not statistically significant ($p > 0.05$). The individual differences, however, spanned from $-0.48$ g/L to 1.09 g/L. The mean BAC estimated from the second void was $2.06 \pm 0.78$ (± SD), which was less than the actual mean BAC observed (2.20 g/L). The mean difference was $0.14 \pm 0.024$ g/L ($p < 0.05$), and the individual differences spanned from $-0.30$ to 0.43 g/L.

Figure 2 shows scatter plots of urine/blood ratio of alcohol and urinary creatinine (lower plot) and also between UAC and creatinine (upper plot). The lack of association is confirmed by the low and nonsignificant correlation coefficients $r = -0.14$ ($p > 0.05$) for UAC and $r = -0.19$ ($p > 0.05$) for UAC/BAC. This lack of association between UAC/BAC ratio and urinary creatinine is more easily seen from the vertical bar graphs in Figure 3.

A small positive correlation was established between urine/blood ratios of ethanol and UAC ($r = 0.41, p < 0.05$), and the relevant scatter plot is shown in Figure 4. The UAC and BAC values were highly correlated both for the first void ($r = 0.94, p < 0.001$) and the second void ($r = 0.96, p < 0.001$) as shown by the scatter plots in Figure 5. The 95% prediction limits for a single new observation are shown, and two outlying values can be identified in the upper plot, which depicts the first urine void. In these individuals, the UAC was less than the BAC at the time of voiding.

Discussion

Ethanol distributes throughout the total body water after drinking, and any binding to plasma proteins appears to be negligible with only trace amounts entering into lipids and bone. Between 1 and 5% of the dose of ethanol consumed is excreted unchanged in urine, sweat, and exhaled air (20). Most of
the ethanol is metabolized in the liver to produce acetaldehyde, and this toxic metabolite is oxidized to acetate, which is then oxidized in peripheral tissues to give the end products carbon dioxide and water (21).

Because only relatively small amounts of ethanol are excreted unchanged as opposed to being metabolized, large increases in the output of urine, such as might result from

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**Table I. Urinary Ethanol (UAC), Urinary Creatinine, and UAC/BAC Ratios (mean±SD) in Two Consecutive Urinary Voids from 40 Drunk Drivers**

<table>
<thead>
<tr>
<th>Urine specimen</th>
<th>UAC (g/L)</th>
<th>UAC/BAC</th>
<th>Creatinine (g/L)</th>
<th>BAC (~g.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>First void</td>
<td>2.88±0.08</td>
<td>1.31±0.209</td>
<td>0.716±0.638</td>
<td>2.22±0.83</td>
</tr>
<tr>
<td>Second void</td>
<td>2.68±0.01</td>
<td>1.20±0.106</td>
<td>0.859±0.727</td>
<td>2.06±0.78</td>
</tr>
</tbody>
</table>

*Estimated* as UAC/1.33 and the actual blood-alcohol concentration (mean was 2.20 g/L).

*The time between first and second void was 69 min (mean±SD).*

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Drinking a large volume of water increases the production of urine by inhibition of the antidiuretic hormone vasopressin and this means a more dilute urine in the subsequent void. Creatinine content, osmolality, and specific gravity of the urine decreased markedly after drinking 500 mL water (3). The resulting diuresis would increase the total quantity of ethanol excreted in the urine, although it still remains a small fraction of the total dose ingested (21). Moreover, regardless of diuresis, the concentration of alcohol in the urine will remain more or less unchanged because alcohol and water are completely miscible and are handled in the same way in the kidney tubules by passive diffusion (18,27).

The concentrations of creatinine in the urine specimens collected from DUI suspects were low compared with the reference values in the prison inmates as might be expected from the well-
known diuretic action of alcohol. In the context of urinary drug screening, the rationale for analyzing creatinine is to control for highly dilute specimens and if necessary report the concentration of illicit drug per milligram creatinine instead of per liter of urine (5,6). There was no association between urinary creatinine, UAC, and the UAC/BAC ratio, so making this type of correction for ethanol concentrations in dilute urine specimens is not necessary.

Lundquist (29) reported a classic study of the urine/blood alcohol relationship, and he measured creatinine in urine samples from DUI suspects (single voids) and attempted to relate the findings to the person’s BAC and diuresis. The highest concentration of creatinine was found in urine from subjects with zero BAC, and the lowest concentration was in those with the highest BAC (29). Lundquist also reported a lack of association between urine flow rate and UAC/BAC ratios based on controlled drinking experiments. When urine flow rate was less than 1.0 mL/min, 1–2 mL/min, or 2–16 mL/min, the mean UAC/BAC ratios were 1.31 (span 1.13–1.47), 1.32 (span 1.12–1.51), and 1.37 (span 1.16–1.51), respectively.

Estimating a person’s BAC from the concentration measured in urine by assuming a population average UAC/BAC ratio has become a controversial subject in forensic toxicology and especially in traffic law enforcement (18). A randomly timed specimen of urine will not necessarily give an accurate estimate of the concentration of ethanol in the blood during the time interval between samplings, and this BAC is estimated from the relationship UAC/1.33. However, translating UAC into BAC for forensic purposes was not necessary in Great Britain because the threshold UAC for prosecution of drunk drivers was defined by statute. Accordingly, a venous blood-alcohol concentration of 80 mg/100 mL was deemed as being equivalent to a UAC (second void) of 107 mg/dL urine, which implies a urine/blood ratio of 1.33:1, although this conversion factor never became an issue for discussion and debate in DUI litigation (19).

The use of urine as a specimen for analysis of ethanol is often criticized because of the risk of ethanol being formed in the specimen after voiding (30). This caution is particularly warranted when urine is obtained from individuals who secrete sugar (e.g., diabetics) or have infections in the bladder or urinary tract (31,32). These two circumstances make it more likely that ethanol could be produced in vitro after voiding or even locally in the bladder by fermentation processes (13,14). The risk of postsampling synthesis of ethanol can be minimized by including NaF in the sampling tubes (at least 1% w/w) and by storing specimens immediately after voiding in a refrigerator at 4°C or, even better, by freezing them (33,34). Various spot tests and color reactions are also available to test for the presence of sugar or bacteria or both in the urine samples before analysis for alcohol.

Recent research has shown that the question of ethanol being formed in the urine in vitro after voiding (e.g., by the action of microbes or yeasts) can be resolved by the analysis of urinary metabolites of serotonin. Studies indicate that the ratio of 5-hydroxytryptophol/5-hydroxyindoleacetic acid (5HTOL/5HIAA) in urine increases appreciably after drinking ethanol unless the ethanol was produced in vitro or in the bladder by the action of yeasts or bacteria using glucose as the substrate (35).

The analysis of urinary ethanol is useful to demonstrate if a person has been drinking alcohol some time before obtaining the specimen and therefore to monitor abstinence. However, the UAC for a randomly timed sample of urine should not be used to estimate the coexisting BAC or to draw conclusions about the degree of alcohol impairment at the time of voiding. Nevertheless, the UAC of a second void 30–60 min after emptying the bladder of old urine will bear a close relationship to the coexisting BAC during the collection period, and this is estimated from the ratio UAC/1.33.

An increase in the glomerular filtration

![Figure 4. Scatter plot of the relationship between urine/blood ratio of alcohol and the urinary alcohol concentration for the first and second voids. The correlation coefficient (r) was r = 0.41 for the whole material and this increased to r = 0.44 when three outliers were eliminated.](https://academic.oup.com/jat/article-abstract/22/3/184/789080)
rate after drinking a large volume of water will dilute the urine but not the concentration of ethanol in the subsequent void. If the UAC was lowered after drinking water and the BAC remained unchanged, one would expect to find abnormally low UAC/BAC ratios coinciding with peak diuresis, but this is not the case (23). Filtering more water in the kidney means filtering more ethanol, so the concentration in the urine is virtually unchanged (27). This is supported by the present findings of a lack of association between urinary creatinine, UAC, and UAC/BAC ratio of ethanol.

![Graph](https://example.com/graph.png)

**Figure 5.** Correlations between urine alcohol and blood alcohol concentration for the first (upper plot) and second urinary voids (lower plot). Two outliers shown on the upper plot suggests that these individuals were still absorbing alcohol at the time of sampling.

**References**


\[ N = 40  \]
\[ UAC = 1.32 \text{ BAC} - 0.04 \]
\[ r = 0.93 \]

![Graph](https://example.com/graph2.png)

**Figure 5.** Correlations between urine alcohol and blood alcohol concentration for the first (upper plot) and second urinary voids (lower plot). Two outliers shown on the upper plot suggests that these individuals were still absorbing alcohol at the time of sampling.


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