COMPARISON BETWEEN THE URINARY ALCOHOL MARKERS EtG, EtS, AND GTOL/5-HIAA IN A CONTROLLED DRINKING EXPERIMENT

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Abstract — Aim: Urinary ethyl glucuronide (EtG), ethyl sulfate (EtS), and the ratio between 5-hydroxytryptophol-glucuronide and 5-hydroxyindole-3-acetic acid (GTOL/5-HIAA) are all suggested as biomarkers for recent alcohol ingestion with longer detection times than measurement of ethanol itself. The aim of this controlled study was to compare the sensitivities and detection times of EtG, EtS, and GTOL/5-HIAA, after a single ingestion of ethanol. Methods: 0.5 g ethanol/kg body weight was ingested by 10 healthy male volunteers in a fasted state. Ethanol, EtG, EtS, and GTOL/5-HIAA levels were measured in urine samples collected during a 45–50 h period. The total amount of ethanol excreted as EtG and EtS was also determined. Results: Urinary EtG, EtS, and GTOL/5-HIAA showed 100% sensitivity as biomarkers for recent drinking. Compared to ethanol testing in urine, the detection times for GTOL/5-HIAA were ∼5 h longer and for EtG and EtS ∼25 h longer. The maximum EtG concentrations were higher than for EtS in all subjects, and a higher fraction of the ethanol dose was excreted as EtG (median 0.019%) compared with EtS (median 0.011%). Conclusions: This study is the first controlled experiment comparing the time-courses for ethanol, EtG, EtS, and GTOL/5-HIAA in urine. In cases where surveillance of alcohol relapse is needed, measurements of urinary EtG and EtS are sensitive and specific alternatives to ethanol testing. The GTOL/5-HIAA ratio is equally sensitive but with a much shorter window of detection.

INTRODUCTION

There are many situations where monitoring of a person’s alcohol use is desirable. For this purpose, biomarkers of acute and chronic alcohol consumption may be useful. The alcohol biomarkers can be divided into two main groups: those used for detection of sustained heavy drinking (long-term markers) and those used to spot recent ingestion (short-term markers) (Helander, 2003). The short-term markers can reveal even a single intake of alcohol, which may be relevant in alcohol-dependent patients on withdrawal treatment (detection of relapse), in workplace testing, or in programs controlling drinking among pregnant women. The standard way to detect alcohol ingestion or monitor abstinence is by measurement of ethanol in breath, blood, or urine. Because ethanol is cleared fairly rapidly from the body, this method is limited to detecting only very recent drinking (Jones, 1992), and there is a need for sensitive short-term biomarkers with longer detection times.

Ethyl glucuronide (EtG) is a nonoxidative minor metabolite of ethanol formed by glucuronidation, catalyzed by UDP-glucuronosyl transferase (Foti and Fisher, 2005). Numerous studies have indicated that the presence of EtG in a urine sample is a specific and sensitive indicator of recent alcohol ingestion, with a detection time spanning up to several days after drinking large amounts (Schmitt et al., 1995; Wurst et al., 2002; Dahl et al., 2002; Sarkola et al., 2003; Wurst et al., 2003a; Skipper et al., 2004). EtG testing is commercially available in the United States and some European countries. Another nonoxidative minor ethanol metabolite, ethyl sulfate (EtS) (Helander and Beck, 2004), is formed by sulfate conjugation through the action of cytosolic sulfotransferase. EtS seems to have similar potential to EtG as a relapse marker (Helander and Beck, 2005; Wurst et al., 2006).

A third short-term alcohol biomarker is a ratio between serotonin metabolites (Beck and Helander, 2003). During ethanol metabolism, the metabolism of serotonin is shifted from formation of 5-hydroxyindole-3-acetic acid (5-HIAA) toward 5-hydroxytryptophol (5-HTOL), and the 5-HTOL/5-HIAA ratio therefore increases appreciably (Helander and Jones, 2002; Sarkola et al., 2003). A recently developed direct method for measuring 5-HTOL-glucuronide (GTOL), the main urinary metabolite of 5-HTOL in urine, yielded similar results as measurement of free 5-HTOL following enzymatic hydrolysis (Stephanos et al., 2005).

A number of studies have investigated the urinary kinetics of one or two of EtG, EtS, and 5-HTOL/5-HIAA (Helander et al., 1996; Sarkola et al., 2003; Borucki et al., 2005; Wurst et al., 2006), but no controlled experiment comparing all three alcohol biomarkers has been published. The aim of this controlled drinking study was to compare the sensitivities and detection times of ethanol, EtG, EtS, and GTOL/5-HIAA in urine after a single oral dose of ethanol, and to determine how much of the administered dose is excreted as EtG and EtS.

MATERIALS AND METHODS

Study protocol

Ten healthy, male volunteers with a median age of 24 years (range 21–46) and a median body mass index of 23.9 kg/m² (range 20.1–28.3) participated in a controlled experiment. According to self-report, they were social drinkers with a median intake of 32.5 standard drinks (@ 12 g ethanol)/month (range 10–60), but, in agreement with the study protocol, had abstained from alcohol during the week prior to the study. Exclusion criteria were somatic or psychiatric illness and regular use of medication.

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After an overnight fast, the participants signed in for the study at 07.00 a.m. Ethanol (Absolut vodka, 40% v/v ethanol in water) was consumed over a 15-min period at a fixed dose of 0.5 g/kg body weight. Urine collections were made before the start of drinking, and then at every voiding until 45–50 h after the start of drinking. This corresponded to approximately once every 2 h for the first 8 h after intake, and thereafter at variable intervals. The participants stayed at the study site during the first 15 h, while subsequent urine collections were made at home. The total volume of every urine collection was determined, and 20-ml aliquots were stored at 4°C until taken for analysis of ethanol, EtG, EtS, GTOL/5-HIAA, and creatinine.

A standardized meal consisting of bread, cheese, ham, and water was consumed at ~2 h and at ~7 h after the start of drinking, and a third meal consisting of pizza and water was consumed after 12 h. The participants were not allowed to consume any other food or drink, or smoke cigarettes, during the first 24 h of the study. For the rest of the study period, ingestion of alcohol was not allowed, but there were otherwise no dietary restrictions.

The subjects gave informed consent and the study protocol was approved by the National Committee for Research Ethics in Norway and the Directorate for Health and Social Affairs.

Analytical methods
Ethanol was measured by headspace gas chromatography with flame ionization detection (Kristoffersen et al., 2006). The lower limit of detection (LOD) for ethanol in urine was 0.005 g/l and values above this limit were reported as positive. The creatinine concentration was determined according to a previously published method (Foster-Swanson et al., 1994).

The urinary concentrations of EtG and EtS were determined using a liquid chromatography–mass spectrometry (LC-MS) method (Stephanson et al., 2002). The LOD for EtG and EtS were 0.1 mg/l and values above this limit were reported as positive. The urinary GTOL/5-HIAA ratio was determined using a direct LC-MS method (Stephanson et al., 2007). For GTOL/5-HIAA, an administrative cutoff (15 nmol/µmol) was used and values above this level were reported as positive.

Statistics
All data was handled using the Kinetica (version 4.4) pharmacokinetic program. Statistical parameters were calculated using SPSS (version 14.0).

RESULTS
All urine samples collected before ingestion of ethanol were negative for ethanol and GTOL/5-HIAA, while one subject had a low concentration of EtG (0.19 mg/l) and EtS (0.33 mg/l). This subject reported being abstinent from alcohol during the week prior to the study, but admitted a heavy intake (~215 g ethanol) 8 days before entering the study.

Unlike ethanol, EtG and EtS are influenced by urine dilution and thereby related to the urinary concentration of creatinine (Dahl et al., 2002; Bergstrom et al., 2003; Helander and Beck, 2005). The qualitative values were therefore normalized to the creatinine concentration of the sample. The detection times for EtG and EtS were calculated using the last uncorrected value above the LOD.

Table 1. The maximum levels ($C_{\text{max}}$), times to maximum ($T_{\text{max}}$), and total detection times for ethanol, EtG, EtS, and GTOL/5-HIAA in urine after drinking 0.5 g/kg ethanol in a fasted state

<table>
<thead>
<tr>
<th>Ethanol</th>
<th>EtG</th>
<th>EtS</th>
<th>GTOL/5-HIAA</th>
</tr>
</thead>
<tbody>
<tr>
<td>C_{max}</td>
<td>0.6 g/l</td>
<td>60 mg/l</td>
<td>21 mg/l</td>
</tr>
<tr>
<td>$T_{\text{max}}$ (h)</td>
<td>(0.4–0.7)</td>
<td>(47–88)</td>
<td>(14–29)</td>
</tr>
<tr>
<td>Detection timec (h)</td>
<td>5.9 (4.8–7.0)</td>
<td>30 (25–48)</td>
<td>32 (25–39)</td>
</tr>
</tbody>
</table>

The first urine samples collected after the start of drinking were all positive for ethanol, EtG, EtS, and GTOL/5-HIAA. The maximum levels ($C_{\text{max}}$), times to maximum ($T_{\text{max}}$), and total detection times (i.e., time for the last sample with a test result above the LOD for ethanol, EtG, and EtS, and above the administrative cutoff for GTOL/5-HIAA) are shown in Table 1 and Fig. 1. The $C_{\text{max}}$ values (presented in mg/l) were higher for EtG than for EtS in all subjects (median EtG/EtS ratio 2.9, range 1.9–4.6). The $T_{\text{max}}$ for ethanol occurred at ~2 h after drinking, which was ~2–3 h earlier than for the other tests. The $T_{\text{max}}$ for EtG occurred later than for EtS in 7 out of 10 subjects, but this difference was not significant. The total detection times for both EtG and EtS were ~25 h longer than for ethanol, and ~20 h longer than for GTOL/5-HIAA. There were no significant correlations between the $C_{\text{max}}$ values for ethanol and the detection times for EtG, EtS or GTOL/5-HIAA. The individual excretion patterns for ethanol, EtG, EtS, and GTOL/5-HIAA are shown in Fig. 2.

The sensitivity of the different tests to detect the actual ingestion of alcohol at different time points is shown in Table 2. After 4 h, ethanol, EtG, EtS, and GTOL/5-HIAA showed 100% sensitivity. After 20 h, EtG and EtS were still positive in all subjects, while no participants had a positive GTOL/5-HIAA at this time.

On a molar basis, a median of 0.019% (range 0.015–0.024) of the ingested ethanol dose was excreted as EtG and 0.011%
Fig. 2. Individual concentrations of ethanol, EtG, EtS, and GTOL/5-HIAA in urine after ingestion of 0.5 g ethanol/kg body weight. Each line represents one subject (n = 10). The concentrations of EtG and EtS are normalized to a creatinine concentration of 100 mg/dl.
The much higher concentrations of EtG compared to 5-HTOL, ethanol, 78% for 5-HTOL/5-HIAA, and 100% for EtG. The at 15 h after ingestion of 0.4 g ethanol/kg was 56% for urinary 2003), urine samples were only collected at two time points before the start of drinking. In another study (Sarkola et al., 2003; Borucki et al., 2005), detection times will increase after ingesting higher doses. However, whether the low positive initial EtG and EtS values in urine of the subject who admitted heavy drinking 8 days before the study really originated from this intake or was due to denial, or a recent unintentional intake, is unknown.

It was previously shown that neither EtG nor 5-HTOL/5-HIAA accumulated in the body after 1 week of ethanol intake of 0.4 g/kg twice daily (0.8 g/kg/day) (Sarkola et al., 2003). Because the GTOL/5-HIAA ratio was shown to return to baseline much earlier than EtG and EtS, it is likely that the daily alcohol intake must be higher to achieve an accumulation of GTOL. A saturation of UDP-glucuronosyl transferases and sulfotransferases, which will increase the half-lives of EtG and EtS, is possible but has not yet been investigated. Also, the metabolism of ethanol is known to accelerate in heavy drinkers (Kostrubsky et al., 1995), a situation that could lead to lower concentrations of EtG. Even though the accelerated metabolism is mainly due to oxidative pathways (Kostrubsky et al., 1995), nonoxidative pathways may be induced by heavy drinking. One of the UDP-glucuronosyl transferases (UGT1A1) (Foti and Fisher, 2005) that catalyzes formation of EtG has been reported to be induced by ethanol in rats (Li et al., 2000). Other studies found an increased activity of UDP-glucuronosyl transferases after alcohol exposure, but did not separate the different isofoms (Finley et al., 1986; Iwama et al., 1990), while some reported unchanged activities of glucuronosyl transferases (Farinati et al., 1989) and sulfotransferases (Iwama et al., 1990). However, if the activity of the oxidative pathway of ethanol metabolism increases, there will be less ethanol available for the nonoxidative transferases, and subsequently possibly lower concentrations of EtG.

In conclusion, measurement of EtG, EtS, and GTOL/5-HIAA in urine were confirmed as useful tests to detect recent ingestion of ethanol. Positive results were obtained some time after ethanol had been eliminated, with EtG and EtS registering the longest detection times. The GTOL/5-HIAA ratio was equally sensitive in the short run, but showed a much shorter detection window. In cases where surveillance of an alcohol slip or relapse drinking is needed, EtG and EtS in urine offer very sensitive alternatives, or complements, to ethanol testing.

<table>
<thead>
<tr>
<th>Urine test</th>
<th>Frequency of positive tests (%)</th>
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</thead>
<tbody>
<tr>
<td>Ethanol</td>
<td>100 100 100 100 100 100 100</td>
</tr>
<tr>
<td>EtS</td>
<td>100 100 100 100 100 100 100</td>
</tr>
<tr>
<td>GTOL/5-HIAA</td>
<td>100 100 100 100 100 100 100</td>
</tr>
<tr>
<td></td>
<td>0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0</td>
</tr>
</tbody>
</table>

(range 0.010–0.016) as EtS, with a median molar ratio of 1.7 (range 1.4–2.5). The difference was statistically significant (P < 0.001) and due to the higher maximum concentrations of EtG while the terminal values were similar.

**DISCUSSION**

This controlled study demonstrated that measurement of the minor ethanol metabolites EtG and EtS, and the ratio of the serotonin metabolites GTOL/5-HIAA, in all the samples of urine showed 100% sensitivity as alcohol biomarkers after a single moderate ethanol dose (0.5 g/kg). The GTOL/5-HIAA ratio was equally as sensitive as EtG and EtS in the short run, but showed a considerably shorter window of detection. The longer detection times for EtG and EtS are supported by previous studies comparing the sensitivity of EtG with that of 5-HTOL/5-HIAA (Sarkola et al., 2003; Borucki et al., 2005). However, one of these studies (Borucki et al., 2005) was an uncontrolled experiment with the amount of alcohol consumed based on self-report only, and no urine samples were collected before the start of drinking. In another study (Sarkola et al., 2003), urine samples were only collected at two time points (4 and 15 h after ingestion). The sensitivity to detect drinking at 15 h after ingestion of 0.4 g ethanol/kg was 56% for urinary ethanol, 78% for 5-HTOL/5-HIAA, and 100% for EtG. The longer detection window for EtG was suggested to be due to the much higher concentrations of EtG compared to 5-HTOL in the body after drinking (Sarkola et al., 2003). It is known that the EtG test is very sensitive and able to detect intake of even very small ethanol doses (Stephanson et al., 2002; Costantino et al., 2006).

A previous study indicated similar detection times for EtG and EtS (Wurst et al., 2006) but, in contrast to the results of the present work, the authors reported similar maximum concentrations of EtG and EtS. However, the present work and another study (Helander and Beck, 2005) found higher maximum concentrations of EtG than EtS, and both studies also obtained identical median molar ratios of 1.7. It has been demonstrated that wine may contain some EtS (up to ∼40 mg/l) and to a lesser extent EtG (up to ∼4 mg/l) (Politi et al., 2005). The type of alcoholic beverage ingested in the previous study (Wurst et al., 2006) was not stated. It is possible that use of wine could have resulted in higher relative concentrations of EtS. On the other hand, the bioavailability of EtS and EtG after oral ingestion has not yet been determined.

The total amount of the administered ethanol dose excreted as EtG was previously determined to be about 0.02% and, based on the EtG/EtS molar ratio, estimated to be less for EtS (Helander and Beck, 2005). The present results confirmed that about 0.02% of the ethanol dose on a molar basis is excreted as EtG and further showed that 0.010–0.016% is excreted as EtS, the latter value being slightly lower than previously reported for one subject (0.022%) (Wurst et al., 2006). The molar ratio between the amounts excreted as EtG and EtS (median 1.7) was comparable to the molar ratio between their C_max values (median 1.6, range 1.0–2.6). Because of the higher molecular weight of EtG (Mw 222) compared to EtS (Mw 126), the C_max ratio was higher when presented in mg/l (median 2.9).

This study investigated the detection times of EtG, EtS, and GTOL/5-HIAA after a single, moderate ingestion of ethanol. According to previous studies (Wurst et al., 2002; Wurst et al., 2003b; Borucki et al., 2005), detection times will increase after ingesting higher doses. However, whether the low positive initial EtG and EtS values in urine of the subject who admitted heavy drinking 8 days before the study really originated from this intake or was due to denial, or a recent unintentional intake, is unknown.

**REFERENCES**


