Influence of Gilbert's syndrome on the formation of ethyl glucuronide

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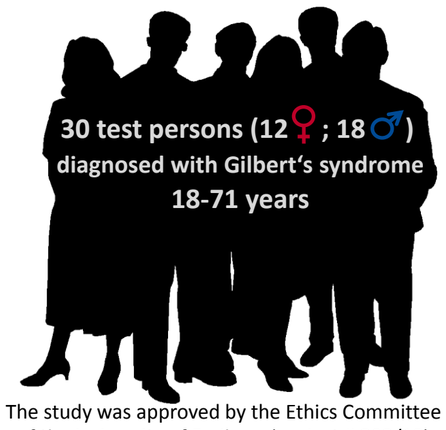
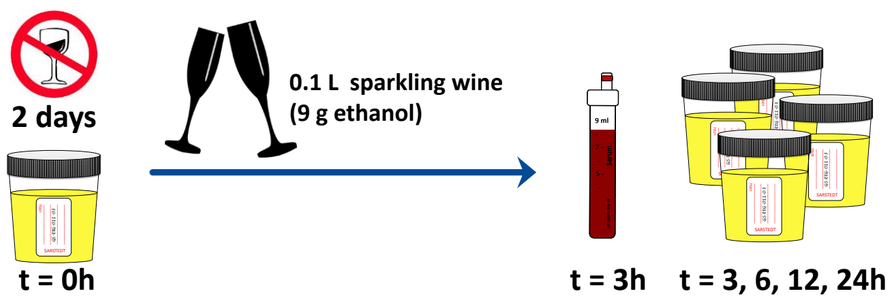
Introduction

EtG is used as a short-term marker for ethyl alcohol consumption to prove abstinence in various settings, e. g. in workplace drug testing programs or before liver transplantations [1]. In drinking experiments, some individuals attracted attention by non-formation of ethyl glucuronide and therefore false-negative assessments. The reason for missing EtG formations has not been identified so far. A possible explanation could be a glucuronidation disorder: Gilbert's syndrome is a rather common congenital metabolic aberration with a prevalence of about 5 %. In most cases the syndrome is asymptomatic; possible symptoms to occur are jaundice and fatigue. Gilbert's syndrome is characterized by a disorder of glucuronidation: the enzyme activity of the isoform 1A1 of the uridine diphosphate glucuronosyltransferase (UGT) is decreased by up to 80 % [2]. UGT 1A1 is among those isoforms stated to form ethyl glucuronide (EtG) following exposure to ethanol [3,4]. A possible influence of the glucuronidation disorder on the formation of ethyl glucuronide was studied in a drinking experiment with participants suffering from Gilbert's syndrome.

Study Design



The study was approved by the Ethics Committee of the University of Freiburg (project # 303/12)



- After two days of abstinence from ethanol and a void urine sample (t= 0h) consumption of 0.1 l sparkling wine
- Urine samples 3, 6, 12 and 24 hours after drinking (t = 3, 6, 12, 24h)
- Blood sample 3 hours after drinking (t = 3h)

Methods

Refrigeration of urine samples prior to analysis.
Quantification of EtG and EtS in all urine samples by LC-ESI-MS/MS (100 µl aliquots, addition of deuterated ISTDs, protein precipitation with methanol, centrifugation, evaporation of the supernatant, reconstitution of the dried extracts with aqueous formic acid) [5].
Determination of creatinine concentrations (photometrical procedure, Jaffé method).

Cool storage of blood samples prior to ethanol determination by HS-GC-FID (Headspace gas chromatography/flame ionization detector, two different separation columns, t-butanol as ISTD).
Immediate analysis of the blood samples in the department of clinical chemistry (blood count, conjugated as well as total bilirubin and liver enzymes).

Results and Discussion

For comparison all EtG and EtS concentrations were normalised to a creatinine value of 100 mg/dL.
The minimum and maximum concentrations are listed below:

All but one t=0h urine sample were negative for EtG and EtS: Only in the non-negative sample a higher concentration of EtS than EtG concentrations was detected (EtG100 0.48 mg/L, EtS100 0.66 mg/L). In all other samples the concentrations of EtG exceeded those of EtS.

From the beginning of research on EtG kinetics, a wide range of metabolization rates was noticed. The marker concentrations in this study also vary markedly. Maximum and minimum concentrations of EtG_{100} and EtS_{100} for t=3h differ by factor 36 and 7.9, respectively.

In 23 test persons the highest concentrations of EtG were observed in the sample taken about 3 hours after drinking. 6 persons showed EtG peak concentrations 6 hours after drinking. In one proband (#11), the highest EtG concentration was measured in the 12 hour post consumption sample, after a decrease of EtG and EtS levels from the 3 hour sample to the 6 hour sample, suggesting a further intake of ethanol.
Only 3 test persons showed the peak concentration of EtS in the sample taken 6 hours after drinking; in all other participants the maximum concentrations were observed in the first urine sampled after drinking (3 h). Figure 1 illustrates these wide inter-individual concentration ranges for EtG.

EtS100 [mg/L]			EtG100 [mg/L]		
	Min	Max		Min	Max
3 h	0.87	6.87	3 h	0.58	18.43
6 h	0.29	4.48	6 h	0.67	13.8
12 h	0	1.19 (8.65)	12 h	0.08	3.39 (13.9)
24 h	0	0.19 (1.25)	24 h	0	0.53 (2.15)

Table 1: Concentration ranges for EtG and EtS. Concentrations in parentheses refer to proband # 11 with a second increase of EtG and EtS concentrations.

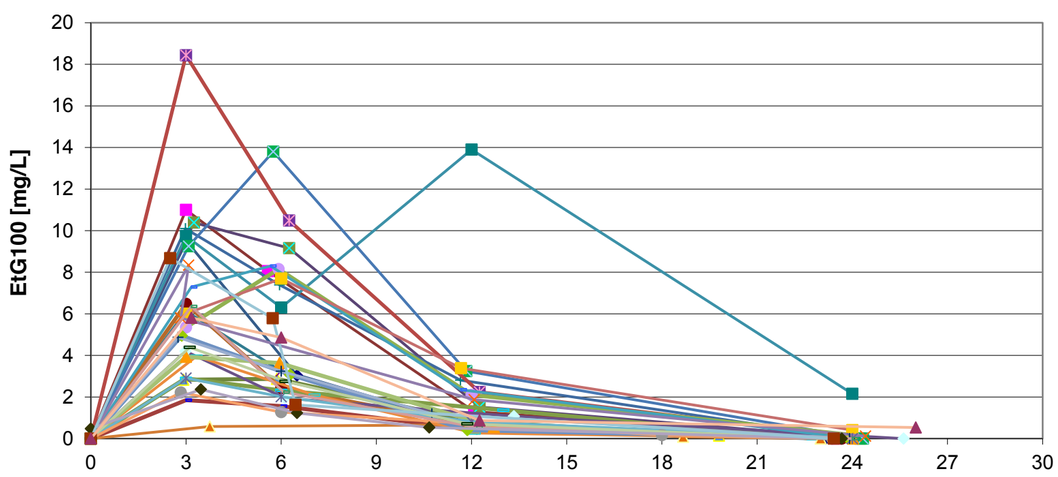


Figure 1: Range of EtG_{100} concentrations

In 6 probands EtG could still be detected after 24 hours without indication of further ethanol consumption. While the maximum concentrations in 4 of these test persons were above the median (6.01 mg/L), the measured maximum EtG_{100} levels in two participants were below the median (see Figure 2). In these two persons, high concentrations cannot be claimed for the long period of detectability. These findings may be ascribed to inter-individual variation in kinetics.

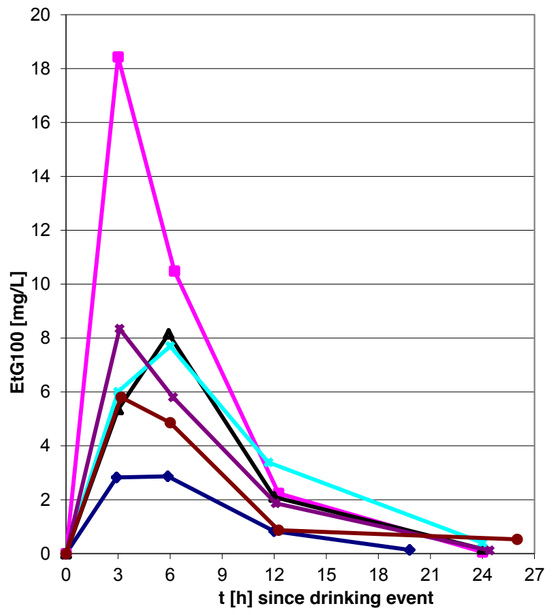


Figure 2: EtG_{100} concentrations in participants with marker detectability 24 hours after drinking

With regard to the initial question, whether Gilbert's syndrome influences the formation of the ethanol glucuronidation product ethyl glucuronide, no dysfunction can be deduced from the results of this study. All participants in this experiment formed and excreted EtG as well as EtS. The maximum EtG_{100} concentrations even exceed those formerly published.

Conclusion

EtG was detected in all participants and seems to be a suitable marker for monitoring abstinence even in persons suffering from the glucuronidation disorder Gilbert's disease. Due to the glucuronidation capability of various isoforms, a mutation of the 1A1 isoform of uridine diphosphate glucuronosyltransferase obviously does not affect the total formation of ethyl glucuronide. Moreover, Gilbert's disease does not serve as suitable explanation for the absence of EtG in urine samples where EtS was detected as observed in previous experiments.

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