Influence of body mass index on hair ethyl glucuronide concentrations

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Influence of body mass index on hair ethyl glucuronide concentrations.

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Abstract

Analysis of ethyl glucuronide (EtG) concentrations in hair is increasingly used to estimate the consumption of alcohol of the prior months. Linear correlations between the amount of alcohol consumed and the concentration of EtG in hair have been reported, and several variables that may influence this correlation have been investigated: e.g., cosmetic hair treatments, gender influences, or hair color. Here, we report on the influence of body mass index (BMI) on the relation between amounts of alcohol consumed and the measured EtG concentrations in hair in 199 participants. Our data shows higher EtG concentrations in participants with high BMI (≥ 25) compared to participants with low BMI (< 25) ($p = 0.001$) upon alcohol consumption, with a consistent difference in EtG between the two groups across a wide range of amounts of alcohol consumed. In overweight and obese participants (BMI ≥ 25), especially with EtG concentrations in hair close to the current cut-off values, the interpretation of hair EtG concentrations may lead to increased false positive rates.

Key words: ethyl glucuronide, EtG, hair, alcohol, body mass index
Introduction

Ethyl glucuronide (EtG) is a minor Phase II metabolite of alcohol that incorporates and accumulates in hair as a direct result of alcohol consumption (Pragst & Balikova, 2006). The quantification of EtG in hair (hEtG) makes it possible to detect and quantify the alcohol consumed over several months, depending on the length of the hair segment being analyzed (for a review, see Crunelle et al., 2014a). A hEtG concentration ≥ 7 pg/mg hair is indicative for repeated alcohol consumption, while a hEtG concentration ≥ 30 pg/mg hair strongly suggests chronic excessive alcohol consumption (consensus of the Society of Hair Testing, http://www.soht.org).

Studies support a linear and positive correlation between hEtG concentrations and the amounts of alcohol consumed over the prior months (Politi et al., 2006; Appenzeller et al., 2007a; Kerekes et al., 2009; Kharbouche et al., 2012; Steward et al., 2013; Crunelle et al., 2014b, 2016a, 2016b). For an optimal interpretation of hEtG results, confounding factors that may influence the incorporation, accumulation, and detection of EtG in human hair have been reported and investigated (Wennig, 2000; Crunelle et al., 2014a). However, thus far, there is little information on the effect of body mass index (BMI) on the correlation between hEtG concentrations and amounts of alcohol consumed.

BMI is a measure of body fat, calculated based on an individual’s length and weight using the equation: mass (kg) / height$^2$ (m$^2$). Based on the calculated BMI, several categories have been formulated to address whether over- or underweight is present (NHI, National Institute of Health). A BMI between 18.5 and 25 kg/m$^2$ is considered optimal, whereas a BMI below 18.5 kg/m$^2$ is indicative for underweight, and a BMI over 25 kg/m$^2$ indicates overweight. A BMI over 30 kg/m$^2$ is associated with being obese.

EtG is produced by glucuronidation of ethanol. It is incorporated into the hair follicles through the blood vessels and through diffusion from sweat. As a result, variability in the
absorption, distribution and elimination of ethanol will lead to variability in blood alcohol concentrations (BAC), and will theoretically influence the incorporated concentrations of hEtG. Alcohol is a polar molecule, and thus preferentially distributes to tissues with high water content. Differences in total body water (TBW) between participants will thus influence alcohol pharmacokinetics (Kent et al., 2012). In a study in 50 volunteers consuming a fixed alcohol dose per kg body weight, the volume of distribution of alcohol decreased with increasing BMI (Maudens et al., 2014). As a result, for people with a similar body weight and administration of a fixed alcohol dose, the alcohol levels in blood will be higher with increasing BMI.

Here, we investigate whether BMI influences the correlation between the amounts of alcohol consumed and the determined hEtG concentrations, and whether BMI should be taken into account when interpreting hEtG concentrations.

**Material and Methods**

**Participants and study samples**

Data of subjects who participated in earlier studies on EtG and alcohol consumption at the Toxicology Laboratory of the Antwerp University between January 2013 and September 2015 (Crunelle et al., 2014b; 2015; 2016a; 2016b) were included. The data inclusion criteria were 1) the participant was aged between 18 and 65 years old, 2) having > 3 cm length scalp hair, 3) the participant provided data on body length and weight (in order to calculate BMI), and 4) the participant provided detailed alcohol intake of the prior 3 months before sample collection using the Alcohol Timeline Follow Back Interview (TLFB; Sobell et al., 1986). Data of participants with bleached hairs, with cosmetically straightened and/or permed hairs, and with gastro-intestinal-, kidney- of liver- pathologies were excluded. Data of participants with hair coloring were included. With these inclusion and exclusion criteria, our database included
samples of 199 participants: 20 teetotalers, 142 consuming alcohol regularly, and 37 alcohol-dependent patients (see Table 1).

*Hair ethyl glucuronide concentrations*

HEtG concentrations were analyzed using a validated method as reported earlier (Crunelle et al., 2014b; 2015; 2016a; 2016b; Cappelle et al., 2015). All analyzed hair samples were first mechanically pulverized, and 30 mg powdered samples were accurately weighted and further analyzed. The majority of the samples (n = 133) were analyzed using gas chromatography–mass spectrometry (GC-MS) in negative chemical ionization mode with 2 ng of EtG-D5 as internal standard and pentafluoropropionic anhydride (PFPA) as derivatization agent (LOD 0.7 pg/mg; LLOQ 2.1 pg/mg). The remaining samples were analyzed with the derivatization agent heptafluorobutyric acid (HFBA), either with GC-MS (n = 36; LOD 0.02 pg/mg; LLOQ 0.08 pg/mg) or with GC-MS/MS (n = 30; LOD 0.05 pg/mg; LLOQ 0.2 pg/mg). In parallel with all abovementioned analyses, several quality control (QC) samples were analyzed. The results of all QC analyses are presented in Figure 1 and all ranged within the QC reference interval provided by the manufacturer (see Figure 1). Coefficients of variation were 13.5 % (QC 1; n = 7), 6.3 % (QC 2; n = 8) and 10.0 % (QC 3; n = 5).

*Alcohol consumption quantification*

Alcohol consumption of the prior 3 months was provided in detail before sample collection using the Alcohol Timeline Follow Back Interview (TLFB; Sobell et al., 1986). The TLFB is well validated in college students, community residents, and in participants in alcohol treatment, to address retrospective alcohol consumption for time frames of ≤ 1 year (Sobell et al., 1986; Carey et al., 2004).

*Statistical analyses*
BMI values were categorized into either: i) four clinical categories: underweight (BMI < 18.5), normal weight (BMI 18.5 – 25.0), overweight (BMI 25.0 – 30.0) and obese (BMI > 30.0, or ii) one group including all participants with BMI < 25.0 (i.e., having an upper BMI corresponding with normal weight) and a second group with BMI ≥ 25.0 (i.e., including overweight or obese participants). In the formal modeling of EtG on alcohol consumption, we opted for the two-group categorization of BMI due to the scarcity of samples in the underweight and obese categories.

Between-group differences (between groups of BMI < 25.0 vs. BMI ≥ 25.0) were tested for significance using student t-tests (for normally distributed data), using Mann-Whitney U tests (for non-normally distributed data), or using Chi-square tests (for dichotomous data).

The relation between amounts of alcohol consumed and hEtG concentrations, and the effect of BMI, was modeled using linear regression. HEtG concentrations were entered as dependent variable, whereas the amount of alcohol consumed, BMI, and the interaction between them were entered as independent variables. Since a regression model on the original variables showed heteroscedasticity and a non-normal distribution of the residuals, the regression model was refitted on log-transformed values, both for the independent (“amount of alcohol consumed”) and dependent (“hEtG concentration”) variable. Checking the fit of this latter model showed a much better fit compared to the model with untransformed variables.

Data are presented as mean ± standard deviation, or as median ± interquartile range (for non-parametric data). A p-value of 0.05 was considered statistically significant.

**Results**

*Participants and study samples*
The included data set is presented in Table 1. The between-group characteristics of the group with BMI < 25 compared to BMI ≥ 25.0 are presented in Table 2.

Since the underweight and obese categories include very few individuals, we decided to recode the BMI into 2 categories, with the value of 25 as cutoff.

Effect of BMI on the relation between hEtG concentration and alcohol consumption.

The findings of a positive and linear correlation between hEtG concentrations with the amounts of alcohol consumed were presented earlier (Crunelle et al., 2014; 2015a, 2015b). Figure 2 confirmed these previous findings in this combined sample (p<0.001; Pearson r = 0.89).

We found a consistent and significant difference in hEtG between the BMI groups. When comparing individuals having consumed similar amount of alcohol, the mean hEtG concentration was significantly higher in the BMI ≥ 25.0 group compared to the BMI < 25.0 group, across the entire range of amounts of alcohol consumed (p<0.001 for the main effect of BMI; Figure 2).

The effect of BMI on hEtG concentrations was also observed with the more classic characterization of participants into the four clinical BMI categories (i.e. underweight (BMI < 18.5), normal weight (BMI 18.5 – 25.0), overweight (BMI 25.0 – 30.0) and obese (BMI > 30.0)).

Discussion

In order to provide reliable hEtG analysis results, it is of importance to identify possible covariates influencing the outcome. For hEtG analysis, several confounding factors have already been investigated, including cosmetic treatment, hair color, and decreased renal function. Hair bleaching and permanent coloring reduces hair EtG concentrations, which may
lead to a false negative interpretation when concentrations are close to the cut-off values (Kerekes & Yegles, 2013; Crunelle et al., 2015). Hair pigmentation was found not to influence hEtG incorporation (Appenzeller et al., 2007b). Also gender had no influence on the correlation between hEtG concentrations and the amount of alcohol consumed (Crunelle et al., 2014b, 2016b). The use of alcohol-containing products, hairspray, gel, oils or grease also does not increase hEtG concentrations (Martins Ferreira et al., 2011; Suesse et al., 2012). Finally, in patients with severe liver disease, hEtG is a sensitive marker to assess retrospective alcohol use (Sterneck et al., 2014), while decreased kidney function influences hEtG levels (Fosen et al., 2016).

The current study contributes to the available knowledge by presenting evidence about the role of BMI on the determined hEtG concentrations. Age was deliberately not included in the regression analysis, due to its high correlation with BMI (p=0.006): in a regression analysis, it is not recommended to include highly correlated variables as independent variable (Neter et al., 1990). The effect of BMI on the hEtG concentrations was modeled using a dichotomous BMI categorization (higher or lower than 25). Due to the small sample sizes of both extreme groups (underweight and obese), the effect could only be statistically investigated in the two-group categorization. The hEtG concentration and amounts of alcohol consumed differed significantly (p<0.001) between both BMI groups, where overweight and obese participants (BMI ≥ 25) were associated with higher concentrations of hEtG compared to under- and normal weight participants (BMI < 25). The study findings are in line with a previous report from Maudens et al. in which was shown that alcohol levels in blood will be higher with increasing BMI. Thus, in the interpretation of hEtG concentrations, it may be advisable to take the participants’ BMI into account to avoid false positive or false negative results. In addition, the study confirms the presence of a BMI-independent positive linear
correlation between hEtG concentrations and the amounts of alcohol consumed, but with a consistent difference in hEtG concentration between individuals with a high and a low BMI.

**Conclusion**

Since hEtG concentrations were found to be influenced by BMI, we recommend BMI should be taken into account when interpreting hEtG concentrations.

**References**


