General information

Testing for chronic excessive alcohol consumption by hair analysis

1. Biochemical basics

After drinking, alcohol is transformed to a small degree on side passes of the metabolism into fatty acid ethyl esters (FAEE) and ethyl glucuronide (EtG) (Fig. 1). Since these minor metabolites still contain the ethyl group of ethanol, they are direct and very specific alcohol markers which cannot originate from other pathological reasons.

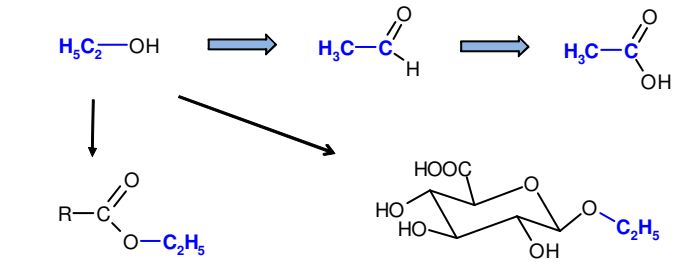

Fig. 1. Fatty acid ethyl esters (FAEE) and ethyl glucuronide (EtG) are minor metabolites of ethanol.

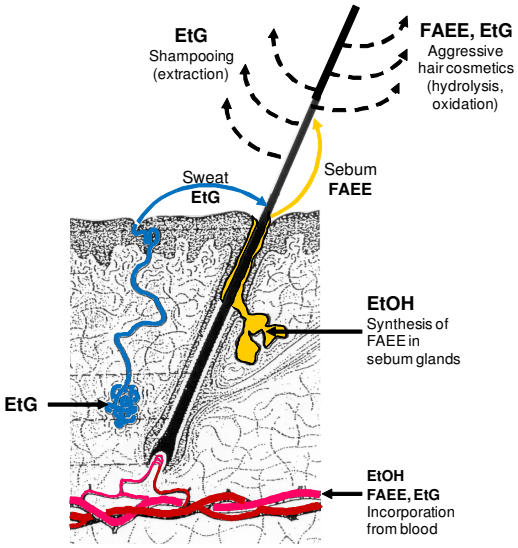

Fig. 2. Incorporation and elimination of FAEE and EtG in hair.

FAEE can be formed in blood and all tissues. EtG is formed almost exclusively in the liver. These markers are deposited in hair during chronically high alcohol consumption. According to the present state of knowledge, FAEE gets into hair mainly by diffusion from the external sebum layer, whereas EtG is deposited into hair mainly from sweat (Fig. 2).

2. Sampling and application for analysis

For collection of the hair sample, the same conditions are valid as in case of testing for illegal drugs: a tuft of hair is tied together with a string at the vertex posterior region of the scalp and cut off as close as possible above the skin. In case of short hair (< 3 cm), it is usually not necessary to fix the hair by a string. In all forensic cases it is advisable to collect two separate samples: one for the analysis and the second is stored untouched for any cases of objection. The sampling can be carried out by one of the nurses cooperating with our laboratory. It is generally recommended to use our hair collection set and the corresponding collection protocol and follow strictly the advices given there. The patient should be interrogated for the frequency of shampooing as well as for the kind and means of hair cosmetics and the frequency of its application. The dry hair samples are preferentially stored in aluminium foil and unambiguously labelled. The proximal end of the sample must be marked. Then, they are sent together with the completely filled-in application form to our laboratory.

In case of too short or totally missing scalp hair, beard, pubic, axillary, chest, arm or thigh hair can be analyzed. These are also cut directly above the skin but need not to be collected as an orderly bundle. The interpretation of the results from these samples is more difficult because of the different growing behaviour and the small amount of reference data. In particular, it must be taken into account, that EtG in pubic hair may be higher than in scalp hair by orders of magnitude.

3. Investigated hair length

According to the standard procedure, the proximal segment 0-6 cm is analysed and interpreted. In case of shorter hair, the whole length is investigated. Since there is no unambiguous relationship between time of alcohol consumption and concentration of FAEE or EtG in the corresponding segments, the analysis of hair longer than 6 cm does usually not lead to improved information. Caused by the accumulation of FAEE on one hand and washing-out effects of EtG on the other, at constant drinking behavior, C_{FSEE} usually increased from proximal to distal whereas C_{EtG} decreases in the same direction (Fig. 3). This must be taken into account in interpretation of deviating length of hair samples.

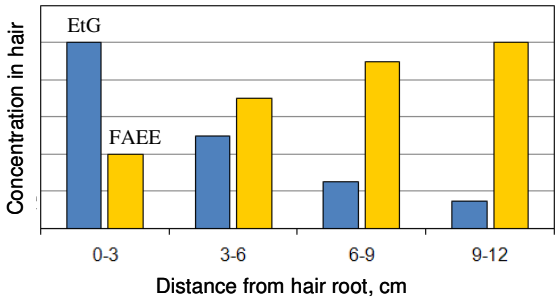

Fig. 3. Schematic course of the concentrations of FAEE and EtG from proximal to distal at constant drinking behavior. For this reason, the interpretation depends on the investigated hair length.

4. Analytical methods

The analytical determination of FAEE and EtG in Haar is performed according to validated specific mass spectrometric procedures using deuterated internal standards. FAEE are determined after removal of the external sebum layer and extracting the hair with a suitable solvent mixture by headspace solid phase microextraction in combination with gas chromatography-mass spectrometry. The four determined esters are ethyl myristate, ethyl palmitate, ethyl oleate and ethyl stearate. The sum of the concentrations of these four esters C_{FAEE} is used for interpretation. EtG is determined from hair after external decontamination and extraction with another solvent by liquid chromatography in combination with tandem mass spectrometry (LC-MS-MS). The range of investigated concentrations of FAEE is from 0.01 to 10 ng/mg and of EtG from 7 to 1000 pg/mg.

5. Interpretation

The interpretation of the concentrations of FAEE and EtG in the proximal 6 cm hair segment is related to the mean drinking behavior during the last months before sampling. Because of the predominant incorporation into hair from sebum or sweat, an unambiguous time-resolved attribution of segmental concentrations to drinking or abstinence periods is not possible. However, longer abstinence leads to low values in the newly grown proximal hair segments.

The scales for interpretation were obtained from the investigation of hair samples from a large number of volunteers with known drinking behavior (teetotalers, social drinkers, alcoholics in withdrawal treatment, and death cases with known alcohol abuse during life time). According to that, the following scale is used:

Drinking behaviour	FSEE (ng/mg)	EtG (pg/mg)
Teetotelers	< 0.2 ng/mg	< 7 pg/mg
Moderate social drinkers	0.2-0.5 ng/mg	< 25 pg/mg
Alcohol abuse	> 0.5 ng/mg	> 25 pg/mg

These are average values. Since the biological processes of formation and incorporation of FAEE and EtG as well as the properties of the hair sample and the hair care and cosmetics vary strongly from subject to subject, deviations in a specific case are possible. Therefore, C_{FAEE} and C_{EtG} should not be interpreted schematically and independent of the information from the whole case. Furthermore, deviating length of the hair sample leads to a change of the interpretation scale (Fig. 3). Cut-off values for alcohol markers in hair are actual matter of discussion in the Society of Hair Testing (SoHT).

6. False positive and false negative results

Because of the flowing change of the drinking behaviour from social drinkers to alcohol abuse and because of the biological variability, false positive or false negative results are generally possible close to the limiting values given above. There is no proportionality between drinking amount and FAEE or EtG concentrations in hair. Therefore, there are also no sharp cut-offs. In addition to that, false positive FAEE values are possible in case of regular application of alcohol containing hair cosmetics (e. g. hair lotion containing 60% ethanol). False positive EtG values are not known until present. On the other hand, false negative values can arise from increased elimination from hair due to hair care and hair cosmetics. Since the more hydrophilic EtG is slowly removed from hair by frequent and intense shampooing, this marker is more sensible to false negative results.

7. Advantage of the combined determination of FAEE and EtG

The combined determination of FAEE and EtG in hair samples increases considerably the accuracy of the interpretation. Both are direct alcohol markers which are formed by different mechanisms, are incorporated into hair on different ways, are differently affected by external interferences and are analyzed with different methods. For these reasons, they complete each other in an optimal way. In Fig. 4, the FAEE und EtG results from 180 hair samples from driving ability examination are shown as interpreted according to the scales given in section 5. Agreeing positive or negative results enable an almost unambiguous interpretation. However, in case of disagreeing results, the absolute value of the concentration of the positive marker is of particular importance.

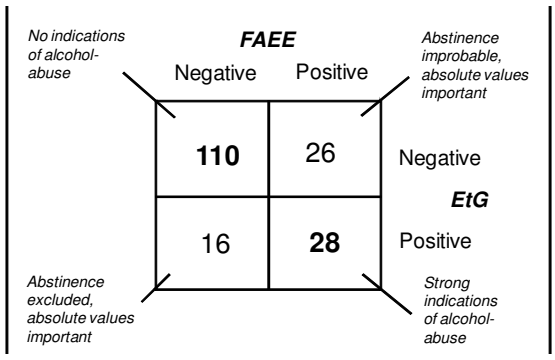

Fig. 4. FAEE and EtG results from 180 hair samples of driving ability examination using the cut-off values 0.5 ng/mg FAEE und 25 pg/mg EtG.

8. References for extended information

1. *F. Pragst, M. Yegles: Alcohol Markers in Hair, In: P. Kintz (Ed.): Analytical and Practical Aspects of Drug Testing in Hair, CRC Taylor & Francis, Boca Raton, FL, 2006, pp. 287-323.*
2. *F. Pragst, M.Yegles: Determination of fatty acid ethyl esters (FAEE) and ethyl glucuronide (EtG) in hair: a promising way for retrospective detection of alcohol abuse during pregnancy? Ther Drug Monit. 30 (2008):255-63. Diese Arbeit kann Ihnen auf Anfrage per E-Mail als pdf-Datei zugesandt werden.*