

FATTY ACID ETHYL ESTERS AND HAIR ANALYSIS: BIOMARKERS FOR ALCOHOL CONSUMPTION AND THEIR POSSIBLE APPLICATION IN THE DIAGNOSIS OF FASD

Daniela Caprara, Graduate Student in Pharmacology, The University of Toronto

A CRITICAL REVIEW of:

“Analysis of fatty acid ethyl esters in hair as possible markers of chronically elevated alcohol consumption by headspace solid-phase microextraction (HS-SPME) and gas chromatography-mass spectrometry (GC-MS).” *Pragst F, Auwaerter V, Sporkert F, Spiegel K. Forensic Sci.Int. 2001;121(1-2):76-88.*

“Fatty acid ethyl esters in hair as markers of alcohol consumption. Segmental hair analysis of alcoholics, social drinkers, and teetotalers.” *Auwaerter V, Sporkert F, Hartwig S, Pragst F, Vater H, Diefenbacher A. Clin Chem. 2001;47(12):2114-23*

“Fatty acid ethyl esters: a novel biologic marker for heavy in utero ethanol exposure: a case report.” *Klein J, Karaskov T, Koren G. Ther.Drug Monit. 1999;21(6):644-6.*

One of the most difficult issues regarding fetal alcohol spectrum disorder (FASD) lies in the diagnosis of less apparent forms of the syndrome where no history of maternal drinking is available and where no physical features have manifested. In the past decade, the use of hair analysis methods to detect and monitor drug use in pregnancy has expanded, becoming the third most fundamental biological matrix used for drug testing after blood and urine¹. One of the most significant advantages of hair analysis involves the wide time window of detection that is not possible with the use of other biological samples. Analytical methods for such substances as opiates, cocaine, and amphetamines have been well established. To date, few methods have been published

describing the use of hair analysis to successfully quantitate alcohol consumption. This lack of research is not due to lack of interest, but rather a lack of potential alcohol markers in hair. Recent investigations conducted by the Pragst and Auwaerter group in Berlin have initiated the promising use of fatty acid ethyl esters (FAEE) in hair as possible markers for elevated alcohol consumption in adults^{2,3}. Their successful analytical method has now opened doors in extending the use of hair testing to identify in utero alcohol exposure, a notion that may change the way FASD is detected and diagnosed.

Ethanol is a small and highly volatile molecule. As such, it does not accumulate appreciably within biological matrices, including hair. FAEE are products of the non-oxidative metabolism of ethanol⁴. Unlike ethanol, they have been found to concentrate in organs commonly damaged by chronic alcohol abuse⁵ and they persist in blood for more than twenty-four hours after significant alcohol consumption⁶. As a result of their hydrophobic nature, FAEE have the potential to enter into the hair shaft and remain for the life of the hair, or until cut⁷. Consequently, FAEE may be suitable long-term markers for identifying and quantifying alcohol use.

Recent work done by Pragst et al. have documented a successful method for the analysis of FAEE in hair³. Briefly, ethyl myristate, ethyl palmitate, ethyl oleate and ethyl stearate were extracted from hair samples using a dimethylsulphoxide (DMSO)/n-hexane mixture, followed by a separation and evaporation of the hexane phase. The remaining extract was then reconstituted in

phosphate buffer and levels of FAEE were quantified using headspace solid-phase micro-extraction (HS-SPME) in combination with gas-chromatography/mass spectrometry (GC-MS). Detection limits for FAEE using this method were between 0.01 and 0.04 ng/mg of hair, with a reproducibility between 3.5 and 16%.

This recent study also involved the analysis of hair samples from alcoholics, social drinkers and teetotalers³. Significant levels of all four FAEE were detected in alcoholic hair samples; ethyl palmitate and ethyl oleate were found in highest concentrations, with means of 1.69 and 2.20 ng/mg, respectively. In contrast, hair samples taken from children and teetotalers failed to yield detectable levels of FAEE. For social drinkers, defined as an alcohol consumption of approximately 30-60g per week, levels of FAEE were much lower than what was seen in alcoholic samples, with maximum ethyl palmitate and ethyl oleate levels of 0.40 and 0.32 ng/mg. Thus, from these results, it follows that the measurement of FAEE concentrations in hair can be used as biological markers for excessive alcohol consumption in adults.

Using segmental hair analysis, it is possible to date the approximate time of drug consumption by determining rate of hair growth along with measuring drug concentrations along the length of the hair shaft. Auwaerter et al. have attempted to determine if segmental hair analysis can be applied in the context of dating alcohol consumption². Using a similar analytical method as described above, segments of hair from alcoholics in treatment were analyzed and compared to hair samples taken from social drinkers and teetotalers. Again, FAEE levels in the samples taken from heavy drinkers were significantly higher than those found from the hair of social drinkers and non-drinkers. In almost all cases, segmental concentrations increased from proximal to distal. However, there was no agreement between the self-reported drinking histories of the participating alcoholics and the FAEE concentrations along the length of the hair².

Assuming that there was minimal mis-reporting, this study indicated that segmental hair analysis may not be useful in dating alcohol consumption in adults.

Pragst and Auwaerter have shown that the use of FAEE as a hair marker for chronically elevated alcohol consumption is promising. Their method may be valuable in the development of a biological marker for prenatal ethanol exposure in neonates. Since neonatal hair begins to grow at approximately six months gestational age, any exposures within the last three months of pregnancy may theoretically be found in neonatal hair after birth. Currently, FAEE are being used as biological markers in meconium to identify possible in utero exposure to alcohol. We have been able to document significantly higher levels of FAEE in the meconium of neonates of self-reporting heavily drinking mothers⁸. However, because meconium exists only during the first three post-natal days, without maternal testimony, diagnosis of maternal drinking may be missed thereafter. Conversely, neonatal hair collection can occur months after birth, increasing the window of opportunity to confirm in utero alcohol exposure. In a pilot study to evaluate the potential of FAEE quantitative neonatal hair analysis, our group has used the method outlined by Pragst et al. to analyze hair samples from a woman who admitted drinking socially throughout her pregnancy and from her newborn girl for FAEE⁹. Both mother and newborn hair were positive for FAEE, at 2.6 and 0.4 pmol/mg, respectively, indicating that the analytical technique established in Berlin may hold promise in the neonatal hair analysis of FAEE as a biomarker for in utero alcohol exposure.

In establishing a valid method for measuring FAEE levels in neonatal hair, we may be able to confirm pre-natal ethanol exposure without the need of maternal testimony. As such, this will facilitate a physician's ability to properly diagnose FASD and allow for proper intervention at stages where the effects of in utero alcohol exposure may be minimized and/or prevented.

REFERENCES

1. Nakahara Y. Hair analysis for abused and therapeutic drugs. *J.Chromatogr.B Biomed.Sci.Appl.* 1999;733(1-2):161-80.
2. Auwarter V, Sporkert F, Hartwig S, Pragst F, Vater H, Diefenbacher A. Fatty acid ethyl esters in hair as markers of alcohol consumption. Segmental hair analysis of alcoholics, social drinkers, and teetotalers. *Clin Chem.* 2001;47(12):2114-23.
3. Pragst F, Auwaerter V, Sporkert F, Spiegel K. Analysis of fatty acid ethyl esters in hair as possible markers of chronically elevated alcohol consumption by headspace solid-phase microextraction (HS-SPME) and gas chromatography-mass spectrometry (GC-MS). *Forensic Sci.Int.* 2001;121(1-2):76-88.
4. Bearer CF, Gould S, Emerson R, Kinnunen P, Cook CS. Fetal alcohol syndrome and fatty acid ethyl esters. *Pediatr Res* 1992;31(5):492-5.
5. Laposata M. Fatty acid ethyl esters: ethanol metabolites which mediate ethanol- induced organ damage and serve as markers of ethanol intake. *Prog.Lipid Res* 1998;37(5):307-16.
6. Laposata M. Fatty acid ethyl esters: current facts and speculations. *Prostaglandins Leukot.Essent.Fatty Acids* 1999;60(5-6):313-5.
7. Ursitti F, Klein J, Koren G. Clinical utilization of the neonatal hair test for cocaine: a four-year experience in Toronto. *Biol.Neonate* 1997;72(6):345-51.
8. Klein J, Karaskov T, Koren G. Fatty acid ethyl esters: a novel biologic marker for heavy in utero ethanol exposure: a case report. *Ther.Drug Monit.* 1999;21(6):644-6.
9. Klein J, Chan D, Koren G. Neonatal hair analysis as a biomarker for in utero alcohol exposure. *N.Engl.J.Med.* 2002;347(25):2086.