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Elevated Concentrations of Acetone and Unidentified Compounds in the Breath of Alcohol Abusers

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A highly sensitive gas chromatographic assay was used to measure volatile organic compounds in the breath. Samples of breath from three groups of subjects were studied: normal volunteers (n = 15), acutely intoxicated alcohol abusers (n = 11), and abstinent alcohol abusers (n = 13). Acetone levels were significantly higher in the acutely intoxicated subjects than in the normals ($p < 0.05$) or the abstinent alcohol abusers ($p < 0.05$). Chromatographic peaks arising from 13 unidentified compounds in the breath were observed; two of these unidentified compounds were observed only in the intoxicated and abstinent alcohol abusers. Statistically significance was achieved in only one unidentified peak; elevated levels of this compound were seen in both acutely intoxicated alcohol abusers ($p < 0.01$) and in the abstinent alcohol abusers ($p < 0.01$) when they were compared to normal subjects.

MORE THAN ONE HUNDRED volatile organic compounds have been detected in normal human breath.[1] Assay of these substances requires specialized techniques, since their concentrations are generally too low to be detected in unconcentrated breath by conventional analytic methods such as gas chromatography (GC). We have recently described a highly sensitive new assay capable of detecting nanomolar quantities of ethanol, acetone, and other low molecular weight organic compounds in the breath, using GC with on-column concentration of the sample.[2,3] We report here an application of this assay as a new tool for the investigation of the metabolism of ethanol and other compounds following acute alcoholic intoxication. Volatile compounds were assayed and compared in breath samples from normal volunteers, acutely intoxicated alcohol abusers, and alcohol abusers who had been withdrawn and abstinent for at least 2 weeks.

MATERIALS AND METHODS

Breath Assay Method

The method has been described.[2,3] In summary, alveolar breath samples (approximately 3–4 liters) were collected into Mylar bags, using a device which shunted the first 0.5 liter of breath into an auxillary sidebag. An internal standard (isopropyl alcohol) was added to the bag before the collection. The bag was reheated in the laboratory to volatilize the organic compounds in the breath, and the sample was pumped from the bag through a GC column cooled to 35°C. At this temperature, organic compounds in the breath were adsorbed to the resin packing of the column, while the nitrogen and oxygen passed through freely. The GC oven was then heated in order to liberate the organic compounds from the resin; the compounds were assayed by a flame-ionization detector (FID) as they eluted from the column, and recorded as a series of peaks on a recorder. Each peak was categorized according to its area under curve (AUC) and its relative retention time (RRT) (where RRT = elution time of peak/elution time of internal standard). All assays were performed on the day of collection of the breath sample. Peaks arising from ethanol,[4] acetone,[4] isoprene,[5] methanol,[6] and acetaldehyde,[7] as well as a number of unidentified peaks, were regularly observed in the breath. The relative concentrations of compounds eliciting unidentified peaks were determined from their molar ratio (MR) values;

$$\mathrm{MR} = \frac{1}{V} \cdot \frac{\text{AUC unidentified peak}}{\text{AUC internal standard peak}}$$

where V = volume of breath sample in liters.

Since FID response is proportional to the number of ions entering the detector,[8] the MR value was assumed to vary proportionately with the molarity of the compound eliciting the unidentified peak. MR values were used to compare the relative concentrations of the unidentified compounds eluting with the same RRT in different subjects.

Clinical Study

Three groups of human subjects were studied: normal volunteers, acutely intoxicated alcohol abusers, and abstinent alcohol abusers. All breath samples were collected between 8:00 a.m. and 10:00 a.m., after the subject had fasted since midnight. The characteristics of the three groups are shown in Table 1.

Normal Volunteers (n = 15). This group comprised laboratory and medical school workers in good health with no history of alcohol abuse. None had consumed any alcoholic beverages during the preceding 24 hr.

Acutely Intoxicated Alcohol Abusers (n = 11). These subjects were recruited from the Acute Detoxification Ward in the Clinical Pharmacology Section of the Veterans Administration Medical Center, North Chicago. All were studied within 24 hr of admission to the hospital with a diagnosis of acute alcoholic intoxication, and all had elevated levels of ethanol in their breath.

Table 1. Characteristics of Human Subjects

	Normals	Alcohol abusers, acutely intoxicated	Abstinent alcohol abusers
Total (n)	15	11	13
Males/females	10/5	11/0	13/0
Smokers/nonsmokers	0/15	10/11	13/13
Tobacco intake [packs/day; mean (SD)]	0 (0)	1.7 (0.7)	1.3 (0.5)
Age, mean (SD)	36.3 (11.4)	42.6 (6.0)	40.6 (11.9)
Racial group			
Caucasion	11	10	8
Black	0	1	5
Oriental	4	0	0

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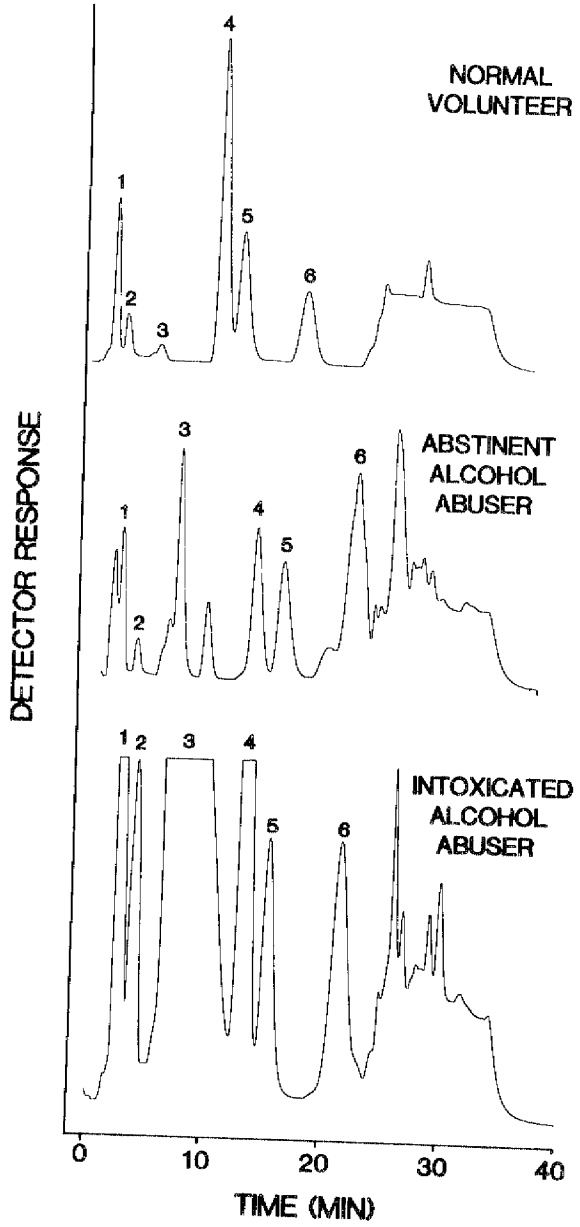

Fig. 1. Gas chromatographic analyses of typical breath samples from a normal subject, an intoxicated alcohol abuser, and an abstinent alcohol abuser. The numbered peaks arise from the following compounds: 1, methanol; 2, acetaldehyde; 3, ethanol; 4, acetone; 5, isopropyl alcohol (internal standard); and 6, isoprene. The remaining peaks are unidentified. Note that the peaks did not elute at identifical times in the three traces, probably due to changes in the performance characteristics of the GC column over time. However, the relative retention time (RRT) of a peak (retention time of peak/retention time of internal standard) remained unchanged in all assays. The "hump" from 20 to 30 min is an artefact of temperature programming.

Abstinent Alcohol Abusers (n = 13). These subjects were recruited from the Alcohol Rehabilitation Unit of the Veterans Administration Medical Center, North Chicago. All had been initially treated in the Acute Detoxification Ward, then transferred to the alcohol-free in-patient environment of the Alcohol Rehabilitation Program, where they had resided for at least 2 weeks before the breath samples were collected.

Human Studies Approval

These studies were approved by the Institutional Review Boards of the Chicago Medical School and the Veterans Administration Medical Center, North Chicago.

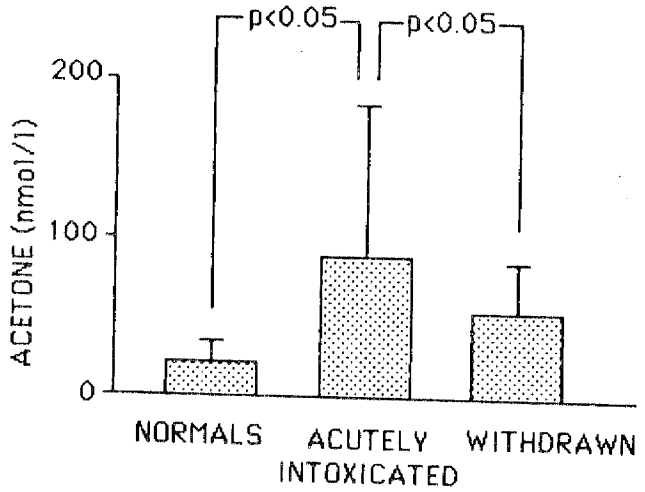

Fig. 2. Concentrations of acetone in breath samples from normal subjects, intoxicated alcohol abusers, and abstinent alcohol abusers. The bar height indicates the mean value for the group, and 1.0 SD.

Statistical Analysis of Data

Molar concentrations of acetone and MR values of the unknown peaks were analyzed by one-way analysis of variance. Where significant differences were observed, data were further analyzed by the Student-Newman-Keuls multiple-range test in order to determine the group of human subjects in which the concentration was elevated.

RESULTS

Typical GC traces of the breath samples obtained from a normal subject, an acutely intoxicated alcohol abuser, and an abstinent alcohol abuser are shown in Fig. 1.

Identified Peaks

Peaks arising from ethanol, acetone, acetaldehyde, isoprene, and methanol in the breath samples were observed in all subjects, and acetone and ethanol were assayed quantitatively.[2,3] The concentration of acetone was significantly elevated in the acutely intoxicated alcohol abusers when compared to the normal subjects ($p < 0.05$) and to the abstinent alcohol abusers ($p < 0.05$) (Fig. 2).

Unidentified Peaks

The MR and RRT values of the unidentified peaks are shown in Table 2. Thirteen unidentified peaks were observed, two of which were not detected in the breath samples obtained from the normal subjects (unidentified peaks 2 and 11, Table 2). The RRT values of the unidentified peaks were similar in all the samples in which they were observed. Statistical significance was achieved in only one unidentified compound: compared to normal subjects, the MR of unidentified peak 4 was significantly elevated in both the acutely intoxicated alcohol abusers ($p < 0.01$) and the withdrawn alcohol abusers ($p < 0.01$) (Table 2 and Fig. 3).

Table 2. Unidentified Peaks: Summary of Findings

Unidentified peak no.*	RRT	Normals		Alcohol abusers, acutely intoxicated		Abstinent alcohol abusers	
		Mean MR	SD	Mean MR	SD	Mean MR	SD
1	0.158	0.002	0.006	0.003	0.006	0.006	0.014
2†	0.210	0.000	0.000	0.020	0.056	0.029	0.047
3	0.442	0.011	0.016	0.003	0.007	0.019	0.030
4	0.642	<0.001	<0.001	0.049‡	0.042	0.075‡	0.050
5	1.222	0.001	0.003	0.010	0.015	0.082	0.185
6	1.562	0.193	0.281	0.153	0.194	0.207	0.192
7	1.645	0.033	0.048	0.233	0.530	0.079	0.127
8	1.703	0.061	0.194	0.010	0.011	0.057	0.156
9	1.764	0.006	0.007	0.033	0.034	0.067	0.113
10	1.822	0.008	0.013	0.026	0.019	0.014	0.031
11†	1.872	0.000	0.000	0.019	0.032	0.029	0.057
12	1.971	0.007	0.018	0.018	0.150	0.045	0.099
13	2.094	0.029	0.044	0.087	0.103	0.019	0.049

* Peak numbers allude to the sequence of elution of unknowns in the gas chromatograph.

† Not observed in normal subjects.

‡ $p < 0.01$, compared to normals.

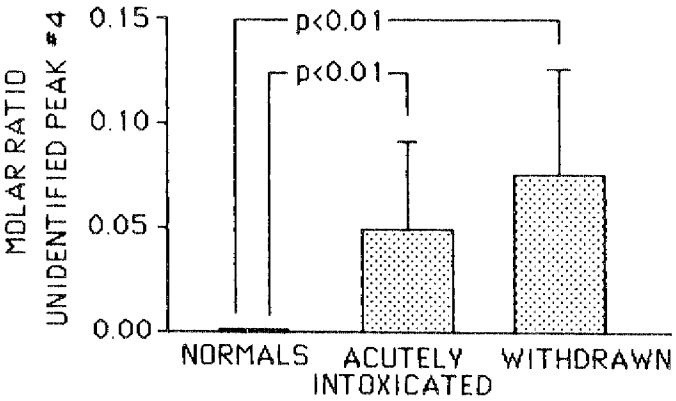

Fig. 3. Molar ratios of unidentified peak 4 observed in normal subjects, intoxicated alcohol abusers, and abstinent alcohol abusers. The bar height indicates the mean value for the group, and 1.0 SD.

DISCUSSION

It has been widely accepted for many years that the metabolism of ethanol in humans is limited to a two-step oxidative conversion, first to acetaldehyde and then to acetate in the liver.[9,10] However, Rutstein et al.[11] have presented evidence for a more complex metabolic pathway for ethanol. They administered distilled spirits to alcoholics and observed increased concentrations of 2,3-butanediol and 1,2-propanediol in the blood. The methodology of their study has been criticized[12]: the diols might have been ingested as ingredients of the alcoholic beverages, and there was no attempt to control the amount or type of alcohol consumed. Nor were their results replicated in another drinking study in which ethanol was administered to "flushing" and "nonflushing" Japanese; no 2,3-butanediol was detected in the blood of any of the subjects, and 1,2-propanediol was not elevated above normal levels.[13]

The existence of alternate pathways of ethanol degradation remains a controversial topic. The unidentified peaks observed in this study might be explained by alternate metabolic pathways of ethanol, but do not confirm their existence. The origins of these unidentified compounds are not known; possible sources may be exogenous (e.g. components of the alcoholic beverages or cigarette smoke), endogenous (e.g., breakdown products of ethanol generated by unrecognized metabolic pathways), or a combination of the two. Further studies are needed in two main areas: first, to identify the chemical composition of these compounds by a more specific assay technique (e.g., mass spectroscopy), and second, to determine whether these compounds are of exogenous or endogenous origin.

In addition, elevated concentrations of acetone were observed in the breath samples from the intoxicated alcohol abusers. The metabolic pathways of ethanol and acetone are known to be linked by at least two mechanisms. Acetone is formed during ketoacidosis,[14] a condition which may complicate an episode of heavy consumption of alcohol.[15,16] Also, ethanol is oxidized in the liver to acetate[9,10] which is then converted to acetyl coenzyme A and acetoacetyl coenzyme A, parent compounds in the biosynthesis of acetone.[14] The observation of elevated acetone in the breath is consistent with the reported elevation of serum 1,2-propanediol in alcoholics consuming distilled spirits,[11] since acetone is converted to 1,2-propanediol by acetone monooxygenase; the activity of this enzyme is stimulated by chronic ethanol ingestion.[17]

We conclude that analysis of volatile compounds in the breath appears to be a promising new tool for the noninvasive investigation of ethanol metabolism in humans. In an earlier report of data from this study, we observed elevated breath ethanol concentrations in the alcohol abusers who had been abstinent for 2 weeks or longer.[18] Analysis of breath volatiles by gas chromatography interfaced with mass spectroscopy might eventually provide a highly sensitive technique for verifying and refuting the existence of alternate pathways of ethanol metabolism.

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