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Limonene in Expired Lung Air of Patients with Liver Disease

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As part of an effort to examine the relationship between chemosensory disturbance and oral chemistry, we analyzed expired lung air samples from a series of 24 patients with liver disease and 24 healthy controls using gas chromatography–mass spectrometry. Compared to samples from controls, lung air from patients with liver disease contained unusually high levels of limonene, a monoterpene that is a major component of the essential oil of citrus fruits (0.1 vs 7.0 μg/20 liters for controls and patients). Only half the patients showed high levels of limonene. Patients with noncholestatic liver disease were significantly more likely to have elevated lung air limonene levels than those with cholestatic liver disease (0.2 vs 13.8 μg/20 liters). Responses to food frequency and dietary behavior questionnaires indicated a pattern of diet selection and food preferences that were consistent with a dietary origin for the limonene in these patients.

KEY WORDS: limonene; liver disease; cholestasis; food preferences; citrus fruit; lung air.

Expired lung air from patients with liver disease can contain unusually high levels of volatile sulfur compounds and aliphatic acids (1–4), and such patients often complain of distortions in taste and smell (5–10). As part of an effort to examine the relationship between chemosensory dysfunction and oral chemistry, we analyzed samples of expired lung air from a series of patients with liver disease for the presence of aliphatic acids and sulfur-containing compounds. In the course of this analysis, it became apparent that lung air from some patients with liver disease unexpectedly contained unusually high levels of limonene, a monoterpene that is a major component of the essential oil of citrus fruits. Here, we report the results of these analyses of expired air and examine information on food intake and preference patterns of these patients to determine whether there may have been a dietary origin for the limonene.

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MATERIALS AND METHODS

Subjects. Twenty-four patients with liver disease and 24 healthy controls who were matched on an individual basis to patients for age, gender, and race were tested. Control subjects, who were obtained through advertisement and from a pool of volunteers at the Monell-Jefferson Taste and Smell Center, had no history of chemosensory dysfunction (eg, anosmia, dysgeusia) or any major medical condition. The patients with liver disease showed no evidence of hepatic encephalopathy or renal disease. The study protocol was approved by the Institutional Review Boards of Thomas Jefferson University Hospital and the University of Pennsylvania.

Patients were classified on the basis of the following clinical criteria:

Primary biliary cirrhosis (PBC; $N = 13$) was diagnosed on the basis of a cholestatic pattern of abnormal liver function tests, presence in serum of antimitochondrial antibody, a compatible liver biopsy, and absence of extrahepatic biliary obstruction.

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Primary sclerosing cholangitis (PSC; $N = 2$) was established by the presence of characteristic obliterative lesions of bile ducts as demonstrated by standard radiologic and histologic techniques.

Alcoholic cirrhosis ($N = 4$) was established by percutaneous liver biopsy and/or clinical evidence of portal hypertension with a history of excess alcohol intake (>80 g/day for at least five years) and exclusion of other causes of cirrhosis.

Chronic active hepatitis ($N = 2$) of the idiopathic type was documented by serum aminotransferase elevations greater than 2.5 times normal for more than six months, hypergammaglobulinemia, presence of autoantibodies, characteristic liver biopsy findings, and exclusion of other causes of chronic hepatitis.

Non-A, non-B hepatitis ($N = 1$) was diagnosed by exclusion on the basis of serologic tests for hepatitis A virus (IgM anti-HAV) and hepatitis B virus [HBsAg, IgM (acute) or IgG (chronic) anti-HBc).

Postnecrotic or cryptogenic cirrhosis ($N = 2$) was diagnosed by liver biopsy or clinical evidence of portal hypertension, consistent liver function test abnormalities, and the absence of an identifiable cause of chronic liver disease.

Lung Air Collection and Analysis. Lung air was examined in 24 patients and 18 healthy controls. Subjects were instructed not to brush their teeth or use mouth wash on the morning that lung air was collected. For collection of samples, subjects were asked to exhale end-expiratory air into a tube connected to a 20-liter Tedlar bag (Cole-Parmer, Inc.) (11, 12). Aliquots of this sample were analyzed as described previously (11, 12) by gas chromatography (for volatile sulfur-containing compounds) and by gas chromatography–mass spectroscopy (for limonene, aliphatic acids). In addition to identification of limonene by its mass spectral fragmentation pattern, the relative chromatographic retention time of the peak corresponding to limonene was compared to the relative retention time of authentic limonene using a mixture of fatty acid ethyl esters (13) (FAEE Index). Quantitation of limonene in lung air was based on the intensity of the molecular ion obtained in the mass spectrum using Incos software. The standard curve for limonene was constructed (see reference 9) by plotting the ratio of the absolute computer-generated intensities of the molecular ion for limonene divided by the intensity of the molecular ion of d_3-anisole generated by 100 ng of this compound against different concentrations of limonene. The concentrations of limonene used to generate the standard curve were 0.05, 0.125, 0.5, 1.0, 10, and 50 μg. Ten injections were performed at each concentration.

Food Preferences and Dietary Behavior. Subjects completed a dietary behavior questionnaire which included open-ended questions about food cravings and a list of 44 food items that subjects rated on a nine-point hedonic scale for perceived pleasantness of taste (8). Some subjects also completed a food frequency questionnaire (14), in which they indicated how many times per week they consumed each of 34 listed food items. Frequency of consumption of six major food groups measured using this questionnaire correlates significantly with frequency of consumption measured with a seven-day diet record (14). Collection of lung air and analysis of samples were performed without knowledge of the subjects' response to the questionnaires.

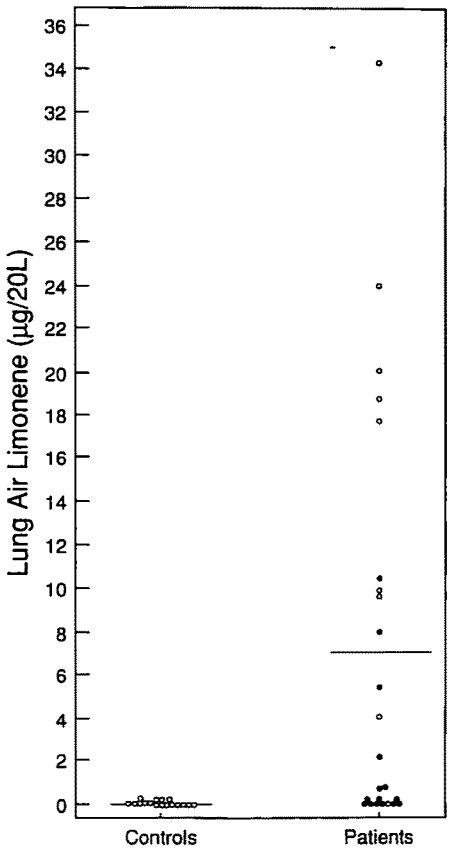

Fig 1. Lung air limonene levels in patients with liver disease and healthy age- and gender-matched controls. Horizontal lines indicate mean of values for each group. In patients, ● = cholestatic disease; O = noncholestatic disease.

Statistics. Statistical evaluations were performed using analysis of variance, except when otherwise noted.

RESULTS

As a group, patients with liver disease had significantly higher levels of lung air limonene than healthy controls [$F(1,40) = 9.34$, $P < 0.005$]; however, on an individual basis, lung air limonene concentrations were markedly elevated in only half the patients (Figure 1). Thus, whereas 12 patients had lung air limonene levels within the normal range (below 1 μg/20 liters), 12 patients showed markedly higher levels, ranging from 2 to 34 μg/20 liters.

The occurrence of an elevated lung air limonene level appeared to be related to the type of liver disease. Patients with noncholestatic liver disease (ie, hepatitis, cirrhosis) were more likely to have high (>1 μg/20 liters) levels of limonene than those

TABLE 1. CLINICAL VALUES IN PATIENTS WITH LOW AND HIGH LUNG AIR LIMONENE LEVELS*

Group	Serum albumin (g/dl)	Serum bilirubin (mg/dl)	Prothrombin time (sec)
Low limonene	3.87 ± 0.20	3.48 ± 2.05	12.11 ± 0.13†
High limonene	3.25 ± 0.26	3.70 ± 0.89	14.17 ± 0.66

*Values are means ± SEM of 11–12 patients.
†Significantly different from patients with high lung air limonene levels, $P < 0.005$.

with cholestatic liver disease (ie, PBC and PSC). Eight of 12 patients with high limonene levels had noncholestatic disease, whereas only one of 12 with low limonene levels had noncholestatic disease ($\chi^2 = 8.7$, $P < 0.01$). Correspondingly, compared to patients in the low-limonene group, patients in the high-limonene group had, on average, a significantly more prolonged prothrombin time and a trend toward lower serum albumin and higher bilirubin levels (Table 1), reflecting the preponderance of noncholestatic (hepatocellular) disease; however, there was considerable overlap between the two groups. Considering only the patients with high limonene levels, those with noncholestatic disease had significantly higher lung air limonene levels than those with cholestatic (PBC) disease [t (10) = 2.15, $P < 0.05$, one-tailed test].

Patients had higher levels of hydrogen sulfide (H_2S) in expired air than did healthy controls [$F(1,31) = 5.88$, $P < 0.05$]. There was no difference in lung air H_2S levels between patients with low as compared with high levels of lung air limonene (Table 2). Methyl mercaptan or dimethyl sulfide was detected in expired air of only three controls and one patient. Similarly, only trace amounts of aliphatic acids (either acetic, propanoic, butyric, isobutyric, or isovaleric) were found in six subjects.

Because limonene is found normally in citrus fruit, we examined data that had been collected on

TABLE 2. HYDROGEN SULFIDE (H_2S) IN EXPIRED AIR OF PATIENTS WITH LOW AND HIGH LUNG AIR LIMONENE LEVELS*

Group	H_2S (ng/10 cc)
Low limonene	
Controls (9)	4.5 ± 0.6
Patients (9)	6.8 ± 1.0†
High limonene	
Controls (7)	4.6 ± 0.7
Patients (10)	6.8 ± 1.1†

*Values are means ± SEM. Number of subjects shown in parentheses.
†As a group, patients were significantly different from controls, $P < 0.05$.

TABLE 3. FREQUENCY OF FRUIT PRODUCT CONSUMPTION PER WEEK IN PATIENTS WITH LOW AND HIGH LUNG AIR LIMONENE LEVELS*

Group	Fresh fruit	Canned fruit	Fruit juice	Total
Low limonene				
Controls (11)	9.9 ± 2.4	0.8 ± 0.4	6.6 ± 1.6	17.3 ± 3.7
Patients (11)	9.6 ± 2.8	0.9 ± 2.8	4.7 ± 1.0	16.1 ± 3.5
High limonene				
Controls (8)	9.9 ± 2.5	1.3 ± 0.9	5.9 ± 0.7	17.3 ± 2.6
Patients (8)	11.5 ± 2.3	3.8 ± 2.6	10.9 ± 1.7†	26.2 ± 3.1†

*Values are means ± SEM. Number of subjects shown in parentheses.
†Significantly different from other groups, $P < 0.05$.
‡Significantly different from controls, $P < 0.05$.

the subjects' dietary habits to determine whether patients with high (>1 μg/20 liters) and low levels of limonene had different food intake practices or preferences. Food frequency data were available for some of the patients with high ($N = 8$) and low ($N = 11$) limonene levels and for their respective controls. Analysis of reported frequency of consumption (Table 3) of fresh fruit, canned fruit, and fruit juice revealed that patients with high limonene levels consumed fruit products significantly more often than their controls [$F(1,14) = 4.7$, $P < 0.05$]. Patients with high limonene in expired air consumed fruit juice significantly more often than patients with low limonene levels or gender- and age-matched controls [$F(3,34) = 3.61$, $P < 0.05$; Ps < 0.05 for individual comparisons]. Because at least 75% of fruit juice consumed is of the citrus variety (15), it seems likely that the increased frequency of fruit juice consumption observed in patients with high limonene levels reflects a greater intake of juices that would contain limonene. There was also a clear trend for a greater frequency of total fruit product consumption in patients with high limonene levels (Table 3). Frequency of total consumption was greater in patients with high limonene compared with controls [$F(1,17) = 4.70$, $P < 0.05$], and showed a marked tendency to be greater in the patients with high as compared with low limonene levels [$F(1,17) = 4.21$, $P < 0.06$].

Responses to the dietary behavior questionnaire provided additional evidence that patients with high limonene levels may be inclined to consume foods with high concentrations of limonene. Among the five patients with the highest limonene levels, three (including the patient with the highest level) reported cravings for fruit (17.8 μg/20 liters), grapefruit (18.9 μg/20 liters), and lemons (34.3 μg/20 liters). Only one patient with low limonene levels

TABLE 4. PREFERENCE RATINGS OF FOODS CONTAINING LIMONENE*

Group	*Bitter-lemon soda*	*Grapefruit*	*Lemonade*
Low limonene			
Controls	4.8 ± 0.6	6.5 ± 0.5	7.0 ± 0.3
Patients	3.5 ± 0.6	6.4 ± 0.4	7.1 ± 0.5
High limonene			
Controls	5.3 ± 0.6	5.9 ± 0.6	7.1 ± 0.2
Patients	6.7 ± 0.6†	7.4 ± 0.4‡	7.3 ± 0.4

*Values are means ± SEM of preference ratings on a nine point scale ranging from dislike extremely (= 1) to like extremely (= 9); 8–12 subjects in each group provided rating for individual food items.
†Significantly different from patients with low lung-air limonene.
‡Significantly different from controls.

reported a craving for fruit, and no controls reported cravings for any particular fruit item. The portion of the questionnaire that contained 44 food items rated for pleasantness included three items that would contain significant amounts of limonene; bitter lemon soda, grapefruit, and lemonade (Table 4). Patients with high lung air limonene levels reported a significantly greater liking for bitter lemon soda than patients with low limonene levels [$F(2,33) = 3.85$, $P < 0.05$; $P < 0.05$ for individual comparison]. This liking did not appear to be based on the bitter taste of the beverage, since ratings for tonic water were similar in patients with low and high limonene levels. Patients with high limonene levels also tended to show an increased liking for grapefruit compared with healthy age- and gender-matched controls ($P < 0.05$). The increased preference for grapefruit has been observed previously in patients with hepatic metastatic cancer (16, 17).

DISCUSSION

We found unexpectedly high levels of limonene in lung air samples of some patients with chronic liver disease, particularly in those with noncholestatic liver disease. Although limonene is used as a fragrance or flavor in a variety of prepared toiletry and food products, the increased frequency of fruit juice consumption reported by patients with elevated lung air limonene levels suggests a natural dietary source for the compound. The increased preference ratings for the taste of at least some citrus products further suggests that these patients are inclined to consume the kinds of foods that contain limonene. However, differences in reported food consumption and preferences were not as marked as those in lung air limonene levels; for example, frequency of fruit juice consumption was increased twofold in patients who had more than a 50-fold increase in lung air limonene levels. These differences might reflect a relatively lower sensitivity of dietary and preference measures; assessing the frequency of consumption and hedonic evaluation of a food does not provide a measure of the amount that is actually consumed. Alternatively, differences in the metabolism and disposal of limonene among patients could account for wide variations in its concentration in lung air.

It is unclear what accounted for the higher lung air limonene levels in patients with noncholestatic liver disease than in those with cholestatic liver disease. Gender and age did not account for the difference, as the distribution of males and females in the two groups were the same (nine females, three males) and the mean ages were similar (48.1 ± 5.4 vs 48.5 ± 3.0 years for patients with high and low limonene, respectively). Examination of medication records revealed no obvious differences between patients with low and high limonene levels. The data on dietary habits suggest that differences in intake of limonene accounted for the differences in lung air limonene levels between the two groups, since the four cholestatic patients with high limonene levels also showed evidence of increased fruit consumption and preference for citrus products. However, given the marked differences in lung air limonene levels among patients and between patients and controls, other factors are likely to be involved. For example, disturbances in the absorption, metabolism, and disposal of limonene might contribute to high limonene levels in expired breath of patients with noncholestatic disease. Because limonene is lipid-soluble, patients with cholestatic disease may have reduced intestinal absorption of limonene. Furthermore, if liver dysfunction in patients with noncholestatic disease impairs the degradation of limonene to nonvolatile or less volatile forms (18, 19), a greater proportion of ingested limonene may be available for disposal via lung air for a more extended period. In fact, on average, measures of hepatocellular dysfunction (serum albumin and bilirubin levels and prothrombin time) were more abnormal in the high-limonene group (statistically significant only for prothrombin time), although there was much overlap in values between the high- and low-limonene groups.

Patients with high limonene levels in expired air did not show elevated levels of other organic compounds examined. Thus, elevated limonene in breath did not reflect a generally greater output of

volatiles. Elevated levels of volatile fatty acids and sulfur-containing compounds have been observed previously in patients with decompensated cirrhosis often associated with fetor hepaticus (1–4). Patients in the present study who apparently had less severe liver disease showed an increase only in lung air H_2S. To our knowledge, H_2S has not been previously detected in patients with liver disease.

Limonene has been used to dissolve gallstones (20) and is reported to have several biological actions, including effects on bile flow (21), the immune system (22), cholesterol metabolism (23), and carcinogenesis (see 24). If patients with high lung air levels of limonene seek out and consume increased amounts of citrus fruits, as the dietary and preference data suggest, it is conceivable that they may be eating for some as yet unidentified medicinal benefit of limonene. Alternatively, lung air limonene may be only a marker of the intake of citrus fruit, which these patients eat because of other real or perceived benefits or because they prefer the flavor. In any case, given the potential pharmacological properties of limonene, further studies of its origin, metabolism, and actions in patients with liver disease are warranted.

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