Myocardial Metabolites of Ethanol

Louis G. Lange and Burton E. Sobel
From the Cardiovascular Division, Washington University, St. Louis, Missouri

SUMMARY. Because of the importance of alcohol-induced heart muscle disease and the obscurity of its pathogenesis, this study was undertaken to determine whether fatty acid ethyl esters, myocardial metabolites of ethanol recently described in our laboratory to be synthesized in cell-free extracts of rabbit myocardium, accumulate in hearts of human subjects exposed to ethanol in vivo. Lipid extracts were prepared from left ventricular samples obtained at necropsy from six subjects who had been exposed to ethanol acutely or chronically. Fatty acid ethyl esters were present in each extract in concentrations ranging from 9 to 115 μM. In contrast, they were consistently absent from analogous samples obtained from hearts of abstainers (n = 5). In parallel studies in experimental animals, we found that fatty acid ethyl esters are formed not only in the heart but also in the pancreas and liver—targets of injury associated with chronic alcohol abuse. These results demonstrate the presence in human myocardium of a novel metabolite of ethanol that potentially may serve as a marker for exposure to alcohol and that could be relevant to the pathophysiology of excessive alcohol consumption leading to cardiac abnormalities. 

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ALCOHOL-INDUCED heart muscle disease afflicts more than 200,000 patients (Bridgen and Robinson, 1964; Fink et al., 1979) and accounts for 3% of all cardiac admissions to city hospitals in the United States (Kramer et al., 1968). Manifestations include accumulation of myocardial triglycerides (Lochner et al., 1969; Regan et al., 1966; Ferrans et al., 1965; Kikuchi and Kako, 1970), decreased β-oxidation of fatty acids (Kramer et al., 1968; Lochner et al., 1969; Regan et al., 1966; Segal et al., 1979), high grade atrial and ventricular arrhythmias, and congestive heart failure (Schwartz et al., 1975; Weisshaar et al., 1977; Bing, 1976; Demakis et al., 1974). These and other abnormalities result from ethanol abuse even in the absence of vitamin and caloric deprivation (Segal et al., 1979; Robin and Goldschlager, 1970; Regan et al., 1977). Nevertheless, elucidation of the pathogenesis or development of specific diagnostic markers of alcohol-induced heart muscle disease has been hampered by the lack of demonstrable metabolism of ethanol by the heart (Lochner et al., 1969) and lack of the identification of specific cardiac metabolites of ethanol with potentially deleterious properties.

We have recently identified metabolites of ethanol formed in extracts of hearts from experimental animals and characterized the products as fatty acid ethyl esters (Lange et al., 1981). In the present study, we found that these specific metabolites of ethanol, fatty acid ethyl esters, accumulate and persist in hearts of human subjects exposed to ethanol in vivo, even after blood alcohol concentration has become undetectable. Thus, metabolites of ethanol have been identified in human hearts for the first time and the relationship of them to developing objective, laboratory markers of significant alcohol exposure and/or to linking them to pathophysiological events in alcohol-induced heart disease is discussed.

Methods

Human autopsy samples were obtained in accord with the guidelines of the Washington University Human Studies Committee from males, 21-54 years of age, either acutely intoxicated with ethanol at the time of death or known to be chronic ethanol abusers. In all cases, death resulted from non-alcohol-related disease or from accidents. Samples from the left ventricle weighing 1-2 g were extracted with acetone, and lipids were separated by thin-layer chromatography (Lange et al., 1981) within 8-12 hours after death. Fatty acid ethyl esters were quantified by gas chromatography by comparing the peak areas with areas associated with added methyl heptadecanoate as standard. Gas chromatography was performed on a Hewlett-Packard model 5810 gas chromatograph. Gas chromatography-mass spectrometry was performed on a Finnegan model 3200 gas chromatograph-mass spectrometer with the use of electron impact ionization.

Formation of fatty acid ethyl esters by homogenates from rabbit organs was assessed by determining the quantity of [14C]ethanol incorporated into fatty acid ethyl esters (Lange et al., 1981). Whole organ homogenates (10% wt/vol) were incubated with [14C]ethanol, 42 mM = 193 mg/100 ml, in 50 mM phosphate, pH 7.4, at 37° for 60 minutes. Lipids were extracted with acetone after addition of [5H]oleate as tracer and [14C]ethyl oleate as carrier and separated by thin-layer chromatography on silica gel developed with petroleum ether-diethyl ether-acetic acid (75:5:1). Fatty acid ethyl esters, Rf = 0.5, were identified by comparisons with standards and eluted from the silica gel with acetone. Solvent was removed and radioactivity determined by conventional scintillation spectrometry. Recovery was between 70 and 90%. Results were expressed as nmol/g wet weight and converted to μM by dividing by 0.8 ml water per gram wet weight. Reproducibility was ±10%.
Results

Identification of Fatty Acid Ethyl Esters in Human Myocardium

To determine whether fatty acid ethyl esters are formed from ethanol in vivo and whether they accumulate in human myocardium, we assayed myocardium obtained at autopsy from subjects known to have been exposed to ethanol. In all samples from each of the six hearts assayed, fatty acid ethyl esters were identified in the gas chromatogram. Ethyl esters of palmitate, stearate, oleate, linoleate, and arachidonate contributed 9, 4, 24, 41, and 19% to the total fatty acid composition of the ethyl esters with retention times being identical (±0.02 min) to those of authenticated standards of fatty acid ethyl esters. In hearts from subjects who had not been exposed to ethanol, no fatty acid ethyl esters were detectable (n = 5). The products were also analyzed by gas chromatography-mass spectroscopy, and the data confirmed the assignment of the product structures as fatty acid ethyl esters. For example, the fragmentation pattern of the most abundant species (Fig. 2), assigned structure being ethyl linoleate, demonstrated the presence of a parent ion peak at 308 m/e, the molecular weight of ethyl linoleate. Peaks diagnostic for ethyl esters were also present at 263 m/e (R - C = 0+) and 88 m/e (-CH₂C—OC₂H₅).

Importantly, fragmentation patterns of the remaining peaks in the gas chromatogram all included prominent mass fragments at 88 m/e, which confirms that all structures are carboxylic acid ethyl esters.

Among the six hearts exhibiting fatty acid ethyl esters, four were from subjects who were acutely intoxicated at the time of death (Table 1). Fatty acid ethyl ester content in these hearts ranged from 13 to 92 nmol/g (17-115 µM). This observation is compatible with formation and accumulation of these metabolites in the heart with rate constants comparable to those observed in experimental animal preparations. There was no direct relationship between the blood ethanol content at the time of death and the fatty acid ethyl ester content of myocardium (Table 1). The two other subjects were chronic alcohol abusers who had no detectable blood levels of ethanol at the time of death. Both had consumed ethanol within 48 hours prior to death. Fatty acid ethyl esters were present in left ventricular samples from both, in concentrations of 7 and 23 nmol/g (9-28 µM) (Table 1). The fatty acid composition of the fatty acid ethyl ester products was somewhat different from that of the esters from hearts of subjects acutely intoxicated at the time of death. Thus, the profile exhibited ethyl esters of palmitate (22%), stearate (3%), oleate (48%), linoleate (8%), and arachidonate (17%). These results indicate that fatty acid ethyl esters formed from ethanol persist in the

![FIGURE 1. Gas chromatogram of fatty acid ethyl esters from the left ventricle of a subject known to be an alcohol abuser. Fatty acid ethyl esters were extracted from left ventricular samples obtained from hearts of subjects who died while acutely intoxicated. Retention times of 1.32, 2.09, 2.39, 2.91, and 5.86 minutes serve to identify ethyl esters of palmitate, stearate, oleate, linoleate, and arachidonate, respectively. An internal standard, methyl heptadecanoate, elutes at 1.53 minutes.](image1)

![FIGURE 2. Neutral lipids were isolated and analyzed by gas chromatography-mass spectroscopy, as described in the text. Depicted here is the mass spectrum of the component having a retention time of 2.91 minutes and representing 41% of the fatty acids present. Its fragmentation pattern is consistent with that of ethyl linoleic acid with M = 308 m/e. (M - 45) = 263 m/e and an m/e = 88 representing -CH₂C—OC₂H₅.](image2)

**TABLE 1**

<table>
<thead>
<tr>
<th>FAEE nmol/g</th>
<th>µM</th>
<th>Blood [ETOH] (mg/100 ml)</th>
</tr>
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<tr>
<td>92</td>
<td>115</td>
<td>43</td>
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<tr>
<td>48</td>
<td>60</td>
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<td>238</td>
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<td>17</td>
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<td>9</td>
<td>0</td>
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<tr>
<td>23</td>
<td>28</td>
<td>0</td>
</tr>
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</table>

Human left ventricular specimens, approximately 1-2 g, were extracted with acetone, and fatty acid ethyl esters (FAEE) were quantified after thin-layer and gas chromatography.
Acid ethyl esters standards and a high-resolution gas chromatographic-mass spectroscopic fragmentation ranging from 9 to 115 JµM (Table 2). Formation of fatty acid ethyl esters is greater in heart than in other organs known to be adversely affected by alcohol exogenously. Third, although endocardial biopsy is not to be considered a routine clinical test, biopsy of adipose tissue is simple and safe even in an alcoholic population. Thus, fatty acid ethyl ester synthetic capacity is greater in heart than in multiple organs, we assessed the synthesis of fatty acid ethyl esters in selected organs from experimental animals. 14C-Labeled ethanol, 42 µM, was incubated with homogenates, 10% (wt/vol), of rabbit pancreas, heart, aorta, liver, adipose tissue, brain, thigh muscle, or with whole blood for 60 minutes at 37°C in 50 mM phosphate buffer, pH 7.4. Lipids were isolated by acetone extraction and thin-layer chromatography. Synthesis of fatty acid ethyl esters was demonstrable in homogenates of pancreas, aorta, heart, liver, and adipose tissue at rates of 56, 42, 40, 23, and 22 nmol of product/g wet weight of tissue (Table 2). In contrast, brain, skeletal muscle, and blood exhibited only low rates of synthesis of fatty acid ethyl esters (4, 2, and 2 nmol/g, respectively). Thus, fatty acid ethyl ester synthetic capacity is greater in heart than in skeletal muscle, is prominent in homogenates of several organs known to be adversely affected by alcohol abuse, and occurs in several locations available for biopsy.

**Organ Specificity of Fatty Acid Ethyl Ester Formation in Experimental Animals**

Since ethanol abuse is associated with damage to multiple organs, we assessed the synthesis of fatty acid ethyl esters in selected organs from experimental animals. 14C-Labeled ethanol, 42 µM, was incubated with homogenates, 10% (wt/vol), of rabbit pancreas, heart, aorta, liver, adipose tissue, brain, thigh muscle, or with whole blood for 60 minutes at 37°C in 50 mM phosphate buffer, pH 7.4. Lipids were isolated by acetone extraction and thin-layer chromatography. Synthesis of fatty acid ethyl esters was demonstrable in homogenates of pancreas, aorta, heart, liver, and adipose tissue at rates of 56, 42, 40, 23, and 22 nmol of product/g wet weight of tissue (Table 2). In contrast, brain, skeletal muscle, and blood exhibited only low rates of synthesis of fatty acid ethyl esters (4, 2, and 2 nmol/g, respectively). Thus, fatty acid ethyl ester synthetic capacity is greater in heart than in skeletal muscle, is prominent in homogenates of several organs known to be adversely affected by alcohol abuse, and occurs in several locations available for biopsy.

**Discussion**

Results of this study demonstrate for the first time that specific metabolites of ethanol accumulate in human hearts after exposure to alcohol in vivo. Structural identification of the isolated lipids seems secure, with comigration of these products in both thin-layer and gas chromatographic systems with respect to fatty acid ethyl esters standards and a high-resolution gas chromatographic-mass spectroscopic fragmentation pattern diagnostic for that of a fatty acid ethyl ester (Silverstein et al., 1974). These neutral lipid products, fatty acid ethyl esters, accumulate in concentrations ranging from 9 to 115 µM (Table 2). Formation of fatty acid ethyl esters from ethanol occurs predominantly through esterification with free fatty acid (Lange, 1982) and to a lesser extent with fatty acyl CoA esters (Polokoff and Bell, 1978), and their accumulation in the heart may contribute to the evolution of alcohol-induced heart muscle disease.

The concentrations of fatty acid ethyl esters found in hearts of patients acutely intoxicated at the time of death bear no direct relationship to simultaneously prevailing blood ethanol concentrations. Many variables, such as the magnitude of integrated exposure to ethanol over time, postmortem changes, the biological half-lives of ethanol and fatty acid ethyl esters, the availability of fatty acid for esterification, and activities of enzyme(s) responsible for formation of the ethyl esters, among others, may be determinants of the amounts of fatty acid ethyl esters present in the heart at a particular interval after exposure to ethanol. The concentrations of fatty acid ethyl esters observed appear to be sufficient to alter cardiac intracellular lipid metabolism based on results obtained with homogenates. For example, 30 µM concentrations of ethyl esters are sufficient to produce 50% inhibition of fatty acyl CoA-cholesterol O-acyl transferase catalyzed esterification of cholesterol (Lange, 1982). Furthermore, 100 µM fatty acid ethyl ester produces 50% inhibition of rabbit ventricle triglyceride lipase (Mogelson and Lange, 1982).

The identification of concentrations of fatty acid ethyl esters as high as 28 mM in the hearts of subjects without detectable blood levels of ethanol at the time of death suggest that the half-life of these lipid products in vivo is relatively long. Possible postmortem artifacts would lead to underestimations of the amount of fatty acid ethyl ester actually present, since no ethanol was present in the samples that might induce simple chemical esterification, and since lipases in the tissue may have cleaved fatty acid ethyl esters present initially. The differences in fatty acid composition between fatty acid ethyl esters found in the hearts of acutely intoxicated subjects compared with those in the hearts of chronic alcohol abusers may reflect differences in the biological half-lives of the individual fatty acid ethyl esters or other not-yet-defined factors. In concert, the results suggest that people who drink regularly and heavily and who maintain high blood alcohol concentrations for prolonged intervals are likely to eviscerate fatty acid ethyl esters in the myocardium for persistent intervals. In such circumstances, effects of these products of ethanol metabolism on intracellular neutral lipid metabolism may be considerable (vide supra).

Additionally, the importance of these metabolites may lie in their use as a marker for recent exposure to ethanol much as glycosylated proteins have been used in assessment of the severity of diabetes mellitus. First, their half-life in vivo is longer than that of ethanol. Second, unlike some ruminants or yeast, there is no endogenous metabolic pathway for production, in humans, of a two-carbon alcohol yet described and, hence, ethyl esters of fatty acids would not appear to be formed unless there has been exposure to ethanol exogenously. Third, although endocardial biopsy is not to be considered a routine clinical test, biopsy of adipose tissue is simple and safe even in an alcoholic population. Thus, fatty acid ethyl esters are extracted and quantified as described in Methods.
esters assayed in adipose tissue may be suitable as a marker for objective assessment of recent alcohol exposure and could represent a step toward diagnosing alcohol-induced end organ damage by inclusive rather than by exclusive criteria. Further studies examining the correlation of fatty acid ethyl esters concentrations with length and duration of alcohol exposure are in progress.

Results of this study document the first identification of specific products of ethanol metabolism, fatty acid ethyl esters, in hearts from subjects exposed to ethanol, and add to the evidence that myocardium can directly metabolize alcohol (Lange et al., 1981; Lange, 1982). The significance of these findings lies in the fact that fatty acid ethyl esters occur in concentrations sufficiently high and persist for intervals sufficiently long to potentially influence myocardial neutral lipid metabolism appreciably. Thus, these metabolites may contribute to biochemical alterations involved in the pathogenesis of alcohol-induced heart muscle disease or could serve as a marker for recent exposure to ethanol; subsequent studies will have to evaluate these possibilities.

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Address for reprints: Louis G. Lange, M.D., Ph.D., Cardiovascular Division, Washington University, School of Medicine, 660 South Euclid Avenue, St. Louis, Missouri 63110.

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References


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L G Lange and B E Sobel

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