SYMPTOMS OF ALCOHOL-INDUCED LIVER DISEASE IN RATS THAT REGULARLY DRINK ALCOHOL

Liver disease is increasingly becoming a health concern in the United States, especially among American Indian /Alaska Native and Hispanic communities. Alcohol abuse is known to induce liver disease, but regular alcohol use, at socially acceptable levels may also cause serious damage. In 2007, we found that rats that drank the equivalent of three standard drinks per day, five days a week had significantly larger livers than those that did not drink alcohol. Our aim was to test whether the liver of rats that drank at this level changed in ways that indicate alcohol-induced liver disease. We used dual energy x-ray measurements to compare the composition, fat mass, muscle fat and percentage fat in the liver of eight male Long Evans rats that never drank alcohol, with the liver of eight, otherwise identically housed and treated rats that regularly drank alcohol. Furthermore sections of the liver were then stained using Gomori's Trichrome stain and examined under a light microscope for symptoms of alcohol induced liver disease. We expected to see compositional changes that were symptomatic of alcohol induced liver disease. If so, this would suggest that regular alcohol use at socially acceptable levels could lead to similar problems in humans.

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Introduction

Alcohol abuse can lead to various forms of alcoholic liver disease (ALD) such as fatty liver disease and cirrhosis of the liver.[1] The liver is responsible for the metabolism of alcohol. The enzyme alcohol dehydrogenase converts ethanol into acetaldehyde, an unstable and toxic substance, and acetaldehyde is then converted to acetate. The hydrogen produced replaces fatty acids as fuel; this increases the quantity of fatty acids and triglycerides in the liver which leads to alcoholic fatty liver disease.[1] In addition, the metabolism of the alcohol increases the activity of the liver, causing the liver to increase in mass.[1] Alcohol can also lead to cirrhosis by damaging liver cells and increasing the ratio of scar tissue to cells.[1]

Moderate levels of alcohol consumption (2 drinks per day, 7 days a week for men) may, however, have cardiovascular benefits. This level of alcohol use appears to reduce cholesterol concentration in the blood stream by increasing levels of high density lipoproteins (HDL) and decreasing the concentration of low density lipoproteins (LDL).[2] It may also help reduce stress.[2] While these possible heart benefits have been widely publicized,[3] alcohol affects can harm much more than the heart. In 2007, we found that 4 rats that drank at levels similar to those proposed for heart health for 18–24 months developed symptoms of ALD. There were, however, no truly equivalent non-drinking rats with which to compare these results.

Our aim was to test if drinking at moderate levels on a regular basis had adverse affects on the livers of laboratory rats. To achieve this we checked the livers of Long Evans rats that had been drinking approximately 15 standard drinks per week for their body weight, for 12 months for symptoms of ALD and compared them with rats that never drank alcohol.

METHODS

Sixteen Male Long Evans rats were obtained from the University of Alaska Anchorage (UAA), Department of Psychology. These rats had all been fed and housed identically. From the age of four months they were used in motivational experiments. For these experiments, they were placed in Skinner boxes for 30 minutes, 5 days per week, where they could obtain 30 minutes of access to a reward solution by pressing a lever.[4] For eight of these rats, the rewards were 10% sucrose (wt/vol.) solutions. These rats never drank alcohol. For the other eight rats, the rewards were 5%, 10%, 15% or 20% ethanol solutions. These rats usually drank the equivalent for their body weight of 3 standard alcoholic drinks (1 standard drink for a human contains 13.7 g of alcohol, eg, 12 oz of beer or 1 shot of spirits[5]) in each 30 minute session.[5] All rats went through their treatment for a year and were euthanized at 16 months of age for reasons unrelated to this project.

The rats were weighed and measured and then dissected in a consistent matter to obtain their livers. Using dual energy x-ray absorptiometry (DXA), we measured the livers' lean mass and fat mass and then calculated the percentage of fat in this organ.[6] We compared the mean values for the rats that drank alcohol and with those for the rats did not using a 1 tailed t-test.

We then froze the livers and sectioned them into 7 μm sections using a

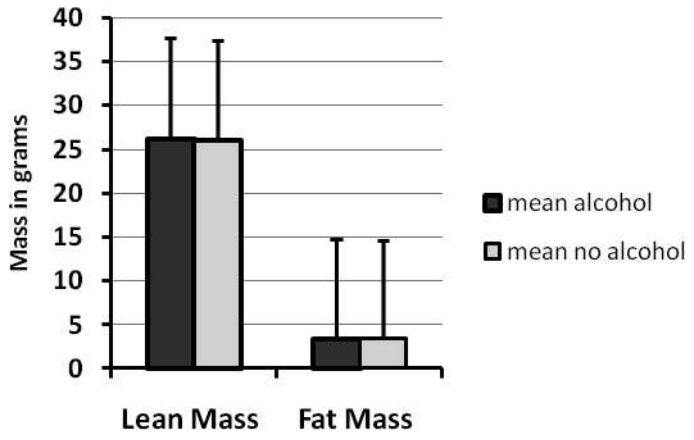

Fig 1. Effect of alcohol use on the composition of the liver. Columns show means, bars show standard errors, *n*=7 for the alcohol treatment, *n*=6 for no alcohol

Cryostat (Microm HM505N). The sections were then transferred to slides and stained using Gomori's Trichrome Stain to distinguish between connective and cellular tissue. We then measured the percentage of connective tissue in the liver sections from each rat and compared the mean percentage values for the two groups using 1-tailed t-test.[7]

RESULTS

The mean overall, lean and fat mass of the liver were not significantly higher for the rats that drank than for those that did not ($P>.05$, Figure 1). However, the mean percentage of connective tissue was significantly higher for the rats that drank alcohol compared to those that did not ($P< .05$, Figure 2).

DISCUSSION

Although two of the three symptoms we tested did not indicate alcohol-induced liver disease, it is worth remembering that these rats drank regularly for only 12 months (euthanized at 16 months of age) and it is being suggested[3] that humans benefit from drinking at such levels for many years. Our results only demonstrate the short-term effects of drinking moderate levels of alcohol. For this short period of time (one year) moderate alcohol consumption did not increase the lean and fat mass of the liver (Figure 1).

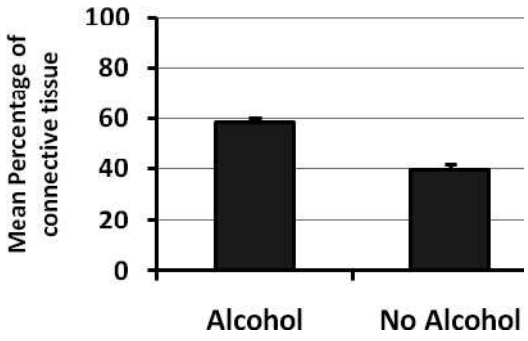

Fig 2. Effect of alcohol use on the mean percentage of connective tissue. Columns show means, bars show standard errors, *n*=7 for the alcohol treatment, *n* =7 for no alcohol

One of the symptoms we tested did, however, indicate damage due to alcohol consumption. The sections from the livers of the alcohol drinking rats showed a significant higher percentage of connective tissue, and thus a significantly lower percentage of cellular material (Figure 2), suggesting that the cell death and scarring typical of cirrhosis had already begun.

The mild symptoms we saw in our rats and the stronger symptoms we saw in older rats suggest that caution should be exercised before recommending this level of drinking as healthy.

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