USING DUAL-ENERGY X-RAY ABSORPTIOMETRY, DXA, TO MEASURE THE EFFECT OF ALCOHOL ABUSE ON THE BONES AND FAT DEPOSITS OF LONG EVANS RATS

Prolonged alcohol abuse in humans is likely to retard muscle development and repair, contribute to obesity and disrupt calcium and bone homeostasis, leading to a reduction in bone mineral density and an increase in the incidence of fragility fracture. Dual energy X-ray Absorptiometry, DXA, which uses a combination of low and high energy x-rays to differentiate between fat, muscle and bone minerals, enables us to quantify these effects under experimental conditions. Our aim was to test the effect of alcohol use on bone mineral composition and bone mineral density in the leg bones of laboratory rats, *Rattus norvegicus* (Long Evans), on the relative proportions of fat tissue, lean tissue and bone minerals in those legs and on the relative proportions of fat and lean tissue in the gastrointestinal tract. We dissected ten control Long Evans rats with two Long Evans rats who were able to self-administer alcohol over the same period. We then used DXA to compare the composition of the legs and gastrointestinal tracts of the treatment and control groups and used power analyses to determine the appropriate sample sizes for future research.

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INTRODUCTION

The medical effects of chronic alcohol use are well documented. It can lead to cancer in the intestines, increased deposition of intestinal fat, and high blood pressure, which, in turn, leads to many other problems in the body.[1] We used laboratory rats, *Rattus norvegicus* (Long Evans), to study the effects of alcohol use on the composition of the forelimb, hind limb, and the intestines. To do this, we used dual-energy x-ray absorptiometry (DXA). The ability of DXA to measure the fat, lean tissue, and bone composition of small mammals has been tested with laboratory mice and is be very precise.[2] Dual-energy x-ray absorptiometry (DXA) uses x-rays with two different energy levels: high-energy x-rays to measure bone mineral composition (BMC) and bone mineral density (BMD) and low-energy x-rays to measure and differentiate between fat tissue and lean tissue. These values can also be used to calculate the total mass of the tissue and the percentage of fat. We examined the effects of regular alcohol consumption on the composition of the BMD, BMC, and the lean tissue and fat tissue content in the limbs and intestines of laboratory rats.

METHODS

We were supplied with 12 Long Evans rats by the University of Alaska Anchorage Department of Psychology. Ten of these rats had never been given alcohol. The other two were allowed to self-administer dilute ethanol solutions of varying concentrations for half an hour each day for approximately six months. The rats never refused to drink. They usually drank in a rapid and sustained manner that left them intoxicated at the end of their sessions. The rats were housed in identical cages, allowed equal and unlimited access to food (rat chow) and water and were, with the exception of the alcohol experiment, treated in a similar manner. The rats were euthanized by the psychology department for reasons unrelated to this project before being provided to us. All of the rats were all dissected identically and in as consistent a manner as possible. The limbs and intestines were measured with an electronic balance to find the wet mass. They were then measured with DXA for the BMD, BMC, lean tissue mass, fat tissue mass, total tissue mass, and the percentage fat. The organs were placed in an oven at 60°C for 24 hours. The masses were taken again after the oven to find the dry mass.

DATA ANALYSIS

We used two-tailed t tests (significance level $=.05$) to test for significant differences between the mean values obtained from the two groups of rats. Where we found no significance, we used power analyses to determine the sample sizes required to obtain a 75% power, assuming a constrained control group size (ie, ≤ 10 non-drinking rats).

RESULTS

The mean percentage of fat in the intestines was significantly greater for the rats exposed to alcohol. The intestinal mass (which included both fat and gut tissue) was also significantly greater for these rats ($P<.05$). No

significant differences between the two groups were found with respect to any aspect of limb composition. The lean, fat, and total mass of the hind limbs did not differ significantly between the two groups. The same was true for the fore limb and for the bone composition of the two limbs (hind limb). Power analysis of our result for the mass of fat in the forelimb, the most likely of the nonsignificant comparisons to yield a difference, showed that increasing the sample size to four would enable us to detect differences as small as .5 g with a power >75%. The other nonsignificant comparisons either already had a power >75% to detect meaningful differences or only needed one or two additional drinking rats to obtain this.

Discussion

Daily use of alcohol led to a significant increase in intestinal fat but no significant change in limb composition. While the latter result may be the result of small sample size ($n=2$) for the alcohol group, this still indicates a pattern of disproportional fat deposition that may be of medical interest, and the sample size is not excessively low given the results of the power analyses.

Study Limitations

A key limitation to this study was the small number of rats ($n=2$) that drank the alcohol. The low number was also disproportionate to the number of rats who did not drink the alcohol and can cause skewed data interpretation.

Implications

Daily use of alcohol increased intestinal fat without causing a significant increase in limb fat. Visceral fat deposition, and in particular disproportionately high visceral fat deposition, is linked to an increased risk of type 2 diabetes in humans. Our results suggest that chronic alcohol abuse can, independent of other factors, increase the risk of this disease.[1]

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