DO RATS THAT FREQUENTLY DRINK ALCOHOL DEPOSIT FATS IN A WAY THAT INCREASES THEIR RISK OF TYPE 2 DIABETES?

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The risk of developing type 2 diabetes is greater in patients with high levels of visceral fat than in equally obese patients with a more even fat distribution. Data from one of last year’s NIDDK student projects suggested that the ratio of intestinal to limb fat was higher in rats that frequently drank alcohol than in those that did not. Our aim was to test whether the ratio of intestinal fat to non-visceral fat was increased by chronic exposure to alcohol. We dissected four Long-Evans rats that regularly drank alcohol and eight Long-Evans rats that never drank alcohol. We used dual-energy X-ray absorptiometry, DXA (Piximus II) to measure the percentage of fat in the intestines and in other organs and structures. The ratio of intestinal fat to non-visceral fat (tail and limbs) deposits was significantly higher (P<.01) for the alcohol-using rats than for the non-drinkers. This suggests a link between alcohol use and increased risk of type 2 diabetes.

INTRODUCTION

Diabetes is a major and growing health problem in the United States. It affects more than 18.2 million people or 6.3% of the population nationwide. Within the United States, 90% of diabetes cases are type 2\(^1\) and the most important risk factor is visceral obesity.\(^2\) Visceral obesity, because of metabolic and anatomical factors associated with intra-abdominal adipose tissue, increases a patient’s risk of type 2 diabetes.\(^2\)

Student researcher Ryan Wilson found that two rats that regularly drank alcohol had significantly higher (P<.05) levels of intestinal fat than 10 rats that never drank alcohol. However, there was no evidence of any significant difference (P>.05) between levels of limb fat for the two groups of rats.\(^3\) This suggested that constant alcohol use in rats increased the risk of type 2 diabetes. Rats are considered to be a good model for studying the effects of alcohol because they can be trained to drink. Therefore, the effects of alcohol on rats can be observed in a more controlled environment than would be possible for human subjects. Our aim was to test whether the ratio of visceral to non-visceral fat was higher in rats that drank alcohol frequently than in rats that never drank.

MATERIALS AND METHODS

In this experiment, 12 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 three 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 3 seconds of access to a reward solution by pressing a lever.\(^4\) For 8 of the rats, the rewards were 10% sucrose (wt/vol.) solutions. These rats never drank alcohol and were all 12 months old when they were euthanized. For 4 of the rats, the rewards were 5, 10, 15 or 20% ethanol solutions. The rats usually drank the equivalent for their body weight of three 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. One of these rats was 24 months old when he was euthanized. The other three were 18 months old. The rats were euthanized for reasons unrelated to this project in June and July 2006. The heart, liver, stomach, spleen, intestines (together with the associated membranes and connected fatty tissue), tail, fore limbs, and hind limbs were dissected and removed from each rat in a consistent manner. They were weighed with a laboratory balance; we measured the percentage of fat in the soft tissue of each component using DXA. We used 2-sample 1-tailed t tests\(^6\) to compare the mean percentage of fat in each of the structures we dissected for alcohol and non-alcohol using rats. We divided the fat percentage of the intestines by the fat percentage of each of three non-visceral elements (tail, fore limb, and hind limb) for each rat to obtain ratios of visceral to non-visceral fat.
RESULTS

Regular alcohol use led to significantly higher levels ($P<.05$) of percentage fat in all tissues and structures measured except the liver, heart, and spleen (Figure 1). These three organs were, however, larger in the alcohol-using rats ($P<.05$: liver $27.9 \pm 2.2$ vs $19.6 \pm 1.7$, heart $1.7 \pm 0.1$ vs $1.3 \pm 0.2$, spleen $1.0 \pm 0.1$ vs $0.6 \pm 0.1$) and the overall mass of liver fat was significantly higher for the rats that drank ($P<.01$). Overall fat mass did not differ between the two treatments for heart and spleen ($P>.05$). The most obvious increase in the percentage of fat was seen in the intestines, which contained $33.4\% \pm 0.2$ in the alcohol-using rats compared with $13.9\% \pm 2.1$ in the rats that did not drink. Tissues unassociated with the viscera such as the tail, the fore limb and the hind limb also had higher fat percentages but the differences were smaller ($34.4\% \pm 0$, $29.0\% \pm 1.1$ and $33.8\% \pm 2.8$, respectively for the alcohol-using rats and $29.1\% \pm 0.5$, $26.9\% \pm 1.2$ and $26.9\% \pm 1.2$, respectively for the non-drinkers). The mean ratio of the percentage of visceral (intestinal) fat to the percentage of non-visceral (tail, fore limb, and hind limb) fat was significantly higher ($P<.01$) for the rats that regularly used alcohol ($1.0 \pm 0.1$, $1.2 \pm 0.1$ and $1.0 \pm 0.1$ for intestine vs tail, vs fore-limb and vs hind limb respectively) than for the rats that had never used it ($0.5 \pm 0.1$ for all of the 3 comparisons) (Figure 2).

DISCUSSION

Regular consumption of alcohol caused an increase of fat in most of the body. The only areas where there was no significant increase in percentage fat were the liver, heart and spleen, which all increased in overall mass, presumably as a result of alcohol-induced liver disease. The increase in percentage fat due to alcohol use was much higher for the intestines than for any other region measured (Figure 1) and, as a result, the ratio of visceral to non-visceral fat for rats that used alcohol was more than double the ratio for rats that never used alcohol (Figure 2). The alcohol-using rats were not

Fig 1. Effect of alcohol use on the percentage of fat in different parts of the body. Bars represent means, error bars represent standard errors, sample size is 4 for the alcohol group, sample size is 8 for the no alcohol group, and asterisks (*) indicate significant difference $P<.05$

Fig 2. Effect of alcohol use on the ratio of visceral to non-visceral percentage fat. Bars represent means, error bars represent standard errors, sample size is 4 for the alcohol group, sample size is 8 for the no alcohol group, and asterisks (*) indicate significant difference $P<.01$
merely obese; they were viscerally obese. As the only aspect of the rats lives that differed was the use or non-use of alcohol, our results suggest that humans who regularly consume alcohol at the same level (3 standard drinks a day) are likely to become viscerally obese and thus increase their risk of type 2 diabetes.

ACKNOWLEDGMENTS
The author thanks Dr. Eric Murphy and Dr. Gwen Lupfer-Johnson of the UAA Dept. of Psychology for the rats; NIDDK, Charles Drew University, and UAA’s Della Keats/U-Doc program for financial support. This project was reviewed and approved by the UAA IACUC committee (protocol #2005VanTe3).

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