SYMPTOMS OF TYPE 2 DIABETES IN THE URINE OF RATS THAT REGULARLY DRINK ALCOHOL

The United States faces a major health problem due to the high and rising prevalence of type 2 diabetes. This is a particular concern for minority groups, with American Indians and Alaska Natives being 2.3 times more likely to have type 2 diabetes than non-Hispanic Whites. Type 2 diabetes is strongly correlated with levels of visceral obesity. An NIDDK student project demonstrated in 2006 that Long-Evans rats that regularly consumed alcohol became viscerally obese, suggesting a link between alcohol use and diabetes risk. The aim was to test whether male Long-Evans rats that regularly consumed alcohol would develop diabetic symptoms. We measured the concentration of glucose and ketones in the urine of eight rats that regularly drank alcohol and compared it to that of eight rats that did not. The mean concentration of glucose was always higher for the rats that drank alcohol, but this difference was not statistically significant (p > .05). Similarly, although we saw a trend toward higher ketone levels in the alcohol drinking rats, this trend was not significant. The rats we measured were younger than those in the earlier study and were not yet obese. Given the observed trend, we expect them to develop diabetes symptoms as they age and become viscerally obese. If so, this would suggest that humans who regularly drink alcohol for a prolonged period of time are at increased risk of type 2 diabetes.

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BACKGROUND

An estimated 6% of US residents are currently diagnosed with diabetes; of these an estimated 90–95% have type 2 diabetes. Diabetes is a leading cause for the amputation of lower extremities, cardiac problems, kidney failure, blindness, and is strongly correlated with obesity, in particular, visceral obesity.

Research conducted through the NIDDK high school summer research apprentice program in 2006 found that 18- and 24-month old male Long-Evans rats that had been trained to regularly drink alcohol from the age of 4 months became viscerally obese. The known correlation between visceral obesity and type 2 diabetes in humans would suggest that these rats also developed type 2 diabetes and that humans who drank at a similar level (3 standard drinks per day, 5 days a week) would be at risk of doing so. Our aim was to determine if the urine of male Long-Evans rats that regularly consumed alcohol at this level showed symptoms of diabetes.

Insulin reception is poor in patients with type 2 diabetes. Due to this, glucose uptake by the cells is lower and slower than individuals without diabetes. As a result, the concentration of glucose in the blood is higher and is maintained at a higher level for longer periods of time. Poor insulin reception also slows fatty acid uptake. These fatty acids in the blood undergo ketogenesis and are converted to ketones, as a result the concentration of ketones in the blood also rises. The heightened levels of blood ketones and blood glucose result in high urinary concentrations of both substances, and elevated urinary levels of glucose and ketones are, therefore, symptomatic of diabetes.

METHODS

Sixteen male Long-Evans rats were used for this project. They were the same age and were housed and maintained identically. From the age of four months, eight were conditioned to push a lever for a sucrose (10%) solution reward, these served as the control group. The eight other rats were conditioned to push a lever for an alcoholic reward, (5, 10, 15 or 20% alcohol concentration). The conditioning of the alcohol group required 30 days of food restriction.

During and after training, the rats were placed in Skinner boxes for 30 minutes, five days a week. In these boxes, they were able to gain access to their reward by pressing a lever. This training and treatment mirrored that of the earlier study, as did all the other aspects of the housing and care.

The rats were weighed and their urine was collected every two weeks. Paraffin filled trays were placed under the Skinner boxes for urine collection. The paraffin prevented urine evaporation. Urine was transferred from the trays to specimen jars and stored at -20°C prior to analysis. Glucose concentration was measured using a commercial glucose assay (Glucose Oxidase Reagent Set, Teco Diagnostics) which created a red byproduct, inminooquinone, whose concentration was equal to the glucose concentration, which could therefore be determined by measuring the absorbance of the resultant solution at 500 nm in a spectrophotometer (Genesys 20). Urinary ketone levels were measured by pipetting 50 µL onto the ketone square of a commercial test strip (Uriscan Gen 8, YD Diagnostics) and comparing the new color of the
Fig 1. Rat Weight Note: Sample size is 8 for all of the means. Error bars = Standard Errors

square with a color chart. The mean body weight and the mean urinary glucose concentrations for the treatment group and the control group were compared using 1-tailed, two sample t tests. Thus this difference was presumably due to the food deprivation that was part of the alcohol-drinking rats’ conditioning. As rats kept on the drinking regime should ultimately develop visceral obesity, we expect the difference between these two groups to equalize and the mean urinary glucose and ketone concentrations of the treatment group to surpass those of the control as obesity develops.

Given the trend towards higher urinary glucose and ketone levels (Figures 2, 3) and the expected development of visceral obesity, we expect the rats that drank to develop symptoms of diabetes as they age. This suggests that, as the rats continue to age and continue to drink, there should be a tipping point. At this point, the urinary glucose and ketone levels of the treatment group should increase and exceed normal, healthy concentrations, whereas the glucose and ketone concentrations in the urine of the control group should remain in the normal range. If so, this would suggest that humans who consume alcohol at this level on a regular basis for an extended period of time are at a heightened risk of developing both visceral obesity and type 2 diabetes.

Fig 2. Effects of alcohol and age on urinary glucose concentrations. Error bars = Std Errors

Fig 3A & 3B. Effects of age and alcohol urinary ketone concentrations. “A” shows urinary ketone values for the alcohol drinking rats. “B” shows values for the rats that did not drink

RESULTS

The mean weight of the rats that did not drink alcohol was significantly higher than that of the rats that drank (P<.05) (Figure 1). Body weight did not significantly increase with age for either treatment during the study period (P>.05, Figure 1). The mean concentration of glucose was always higher for the rats that drank alcohol. This difference, however, was not statistically significant (P>.05) (Figure 2) and mean values were always within the normal range for a healthy rat (0-200 mg/dL). Similarly, although higher ketone levels were more common in the urine samples taken from 7- and 7.5-month old rats that drank alcohol than from the urine of the other alcohol-drinking rats or the control rats, the concentration of ketones, in all of the urine samples, were within the normal range for a healthy rat, 0-10 mg/dL. (Figure 3).

DISCUSSION

Although the non-drinking rats were heavier than the rats that drank, the author would like to thank Dr. Eric Murphy, Fung Fung Lin and Sofia Infante as well as the NIH NIDDK STEP UP program, the NIH NCCR SEPA Northstar program, and UAA WWAMI Biomedical program. This project was reviewed and approved by the UAA IACUC committee (protocol # 2007VanTel).

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