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Single Gene Effect on Alcohol Preference in the Zucker Rats

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STINO, F. K. R. AND K. F. A. SOLIMAN. *Single gene effect on alcohol preference in the Zucker rats.* PHARMACOL BIOCHEM BEHAV 47(3) 625-631, 1994.—Zucker obese (ZO), Zucker lean (ZL), and Sprague-Dawley (SD) naive rats of both sexes were used to study the role of the fa/fa gene on alcohol preference. During the first two weeks of this experiment (weeks 1-2) all rats received a 10% alcohol solution ad lib as the only source of liquid. Animals were then given free access to both water and alcohol for the following three weeks (weeks 3-5). Thereafter, rats were deprived of alcohol every other day for two weeks (weeks 6-7), then completely deprived of alcohol for one week (week 8). Finally, unlimited access to both water and alcohol solution was available for the last two weeks of the experiment (weeks 9-10). Results of this study show that when rats were offered both water and alcohol (weeks 3-5), ZL rats consumed significantly more alcohol (2.5-fold) than their littermates, the ZO rats and the SD rats. Similarly, during alternate days of alcohol deprivation (weeks 6-7), ZL rats consumed significantly more alcohol (threefold) than the ZO rats and the SD rats. After a week of alcohol deprivation (week 8), and during the final two weeks (weeks 9 and 10), ZL rats continued to consume significantly more alcohol (2.5-fold) than the ZO group or the SD group. ZL females consumed more alcohol per kg body weight than male ZL rats. Data obtained from this study suggest that in the Zucker rat a single gene can alter the animal alcohol preference.

Zucker Obese Lean Sprague-Dawley Alcohol Preference Alcohol deprivation

ALCOHOLISM is a persistent, repeated pattern of high alcohol intake that may lead to a consequent tolerance, physical dependence, and inability to control this behavior (10). Evidence from biochemical, electrophysiological, and behavioral studies of alcoholics and their offspring indicate the existence of an inherited predisposition to the development of alcoholism (4). However, this predisposition does not fit into a single pattern. There seems to be a substantial degree of etiologic heterogeneity in the alcoholism phenotype with the ultimate manifestation of the disorder dependent on poorly understood gene-environment interactions (4). The extent to which genetics, as opposed to the environment, contributes to alcohol abuse is not completely understood (4).

Various studies have shown genetically determined differences in alcohol preference in rats. One study compared alcohol preference in four strains of rats (8). The Long-Evans strain was found to consume the highest amount of ethanol, and the Fischer-344 strain the lowest; Wistar and Sprague-Dawley rats were intermediate. Given a choice between water and alcohol solution with continual access to both, most animals drink smaller volumes of the alcohol solution if the concentration of alcohol exceeds 5% (10). However, individual and strain variations in alcohol preference have been found in rats and in inbred strains of mice (11).

The genetically obese Zucker rat mutation appeared spontaneously at the Laboratory of Comparative Pathology (Stow, MA) (19). The Zucker rat is not an inbred strain, and obesity is transmitted as a simple Mendelian recessive trait. However, all Zucker obese females and the majority of the Zucker obese males are sterile (19). Therefore, phenotypically normal heterozygotes of both sexes must be mated to obtain these obese animals. Approximately one fourth of the number of the rats in each litter will be obese from such a mating. This gives rise to Zucker lean and Zucker obese littermates (19).

Alcohol-drinking behavior of different selectively bred and inbred strains of rats has been investigated extensively, and the results of these studies indicate that this character is controlled by multiple genes (10). However, the effect of a single gene variation has not been examined for its possible effect on alcohol preference. Therefore, the present study examined the effect of a single gene (fa/fa) on alcohol preference in the Zucker rat and compared it to that of the Sprague-Dawley rat.

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MATERIALS AND METHODS

Three groups of male and female rats were used in this study: Zucker obese (ZO) and their littermates, Zucker lean (ZL), and Sprague-Dawley (SD). The ZO and ZL groups were used to test the effect of a single gene, the fa/fa gene, on alcohol preference. The SD strain was used as a control. Six males and six females from each of the three groups were used. Sprague-Dawley rats were obtained from Harlan Sprague-Dawley (Indianapolis). Male and female ZL (FA/−) and ZO (fa/fa) rats were obtained from Harlan Blackthorn Bicester (Oxon, UK). Animals were four months old when the experiment started. Each rat was placed in an individual rat cage with bedding. Animals were subjected to 12 h of light daily and were kept in a room with a temperature of 21 ± 1°C. Food (Purina Lab Chow) was provided ad lib. The drinking solution varied as described below.

In the experiment designed to measure alcohol preference and alcohol deprivation effect, the protocol was based on the outline described by Sinclair (16). At the beginning of the study, each rat was weighed and the amounts of food (g) and liquid (water or 10% [V/V] alcohol/water in ml) consumed were measured daily. For the first two weeks of the experiment (weeks 1-2), rats received a 10% alcohol solution as their only source of drinking liquid. Following the first two weeks of forced alcohol consumption, rats were given a choice of either 10% alcohol or water in two separate bottles for three weeks (weeks 3-5). The positions of the bottles were alternated daily to eliminate any position preference effect (16). Rats were then deprived of alcohol, but not water, on alternate days for two more weeks (weeks 6-7). This was done to test the effect of alcohol deprivation for one day on alcohol consumption on the following day (16).

After two weeks of alternating days of alcohol deprivation, rats were completely deprived of alcohol for one week (week 8). Animals were then offered alcohol and water simultaneously for two weeks (weeks 9-10). The amount of alcohol consumed by each rat during the first hour was recorded.

Throughout the experiment, the weekly body weight and daily alcohol, water, and food consumption were monitored for each animal. Data obtained were analyzed, and differences within periods were evaluated statistically using the least-squares analysis of variance (ANOVA) method (13). Regression analysis was used to detect differences in the rate of response between periods (13). The significance level was set at 5% and the Scheffè test was used for comparison of means (14). When the results indicated no significant sex effect for any character, data of both sexes were pooled.

RESULTS

During the first two weeks of forced alcohol consumption, all three groups of rats consumed approximately equal amounts of the 10% alcohol solution (Fig. 1). There was no significant difference between groups or sexes. When rats were offered the choice of either the 10% alcohol solution or water (weeks 3-5), significant differences between groups were observed. ZL rats consumed significantly more ($p < .05$) alcohol (2.5-fold) than the ZO or SD rats (Fig. 1).

In order to test the effect of alcohol deprivation for one day on alcohol consumption on the following day, rats were offered alcohol every other day for two weeks. Neither ZO nor SD rats were affected by this treatment (Fig. 1). However, the ZL rats increased their alcohol consumption gradually and more significantly during the second week following the alternate day of alcohol deprivation.

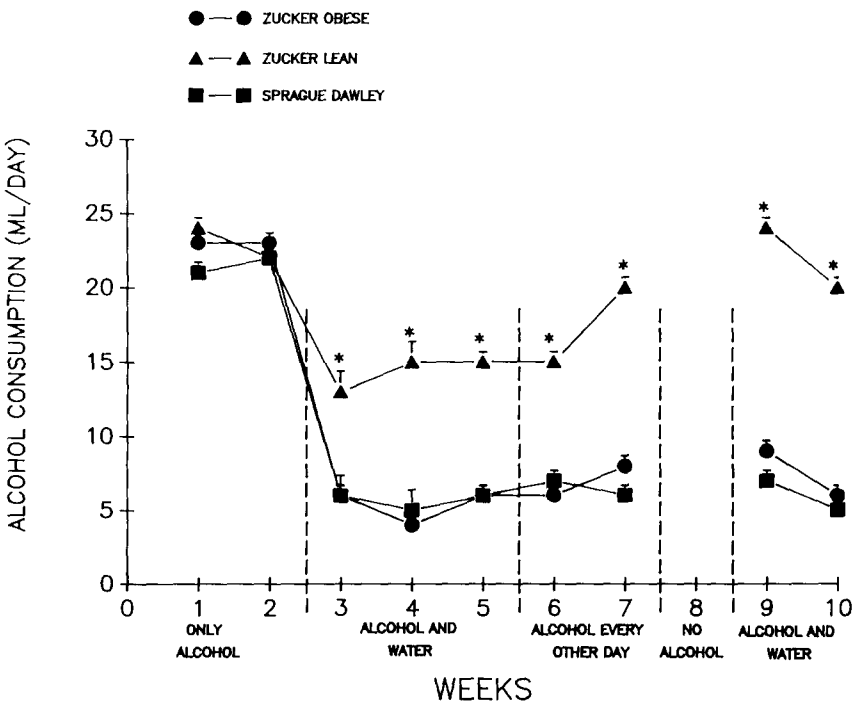

FIG. 1. Alcohol solution (10%) consumption of the different groups of rats over the experimental period. *Zucker lean (ZL) rats consumed significantly ($p < 0.05$) more alcohol than either ZO or SD rats.

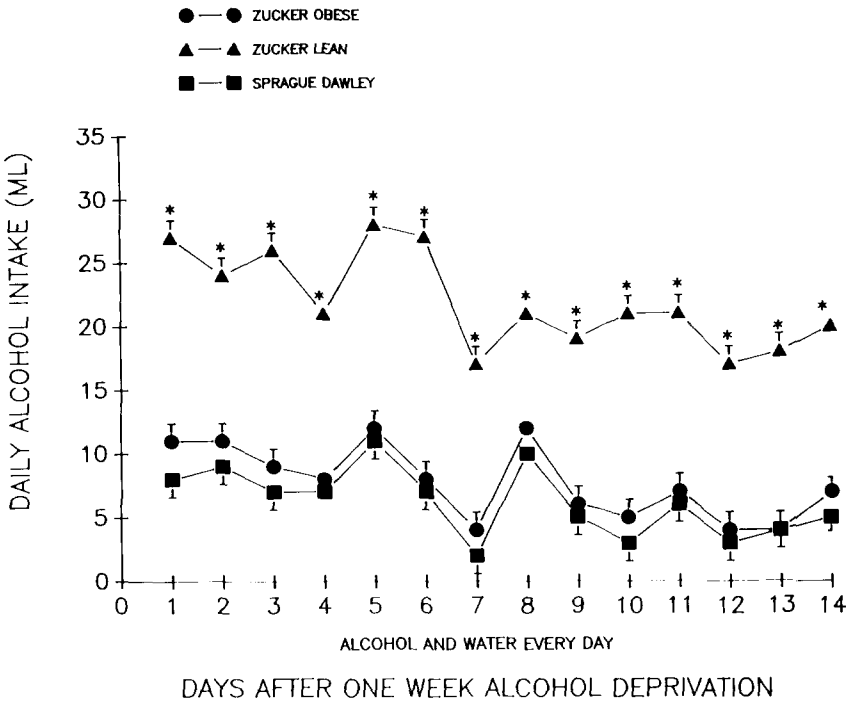

FIG. 2. Daily alcohol solution (10%) intake during weeks 9 and 10 of the different groups of rats. *Zucker lean (ZL) rats consumed significantly ($p < 0.05$) more alcohol than either ZO or SD rats.

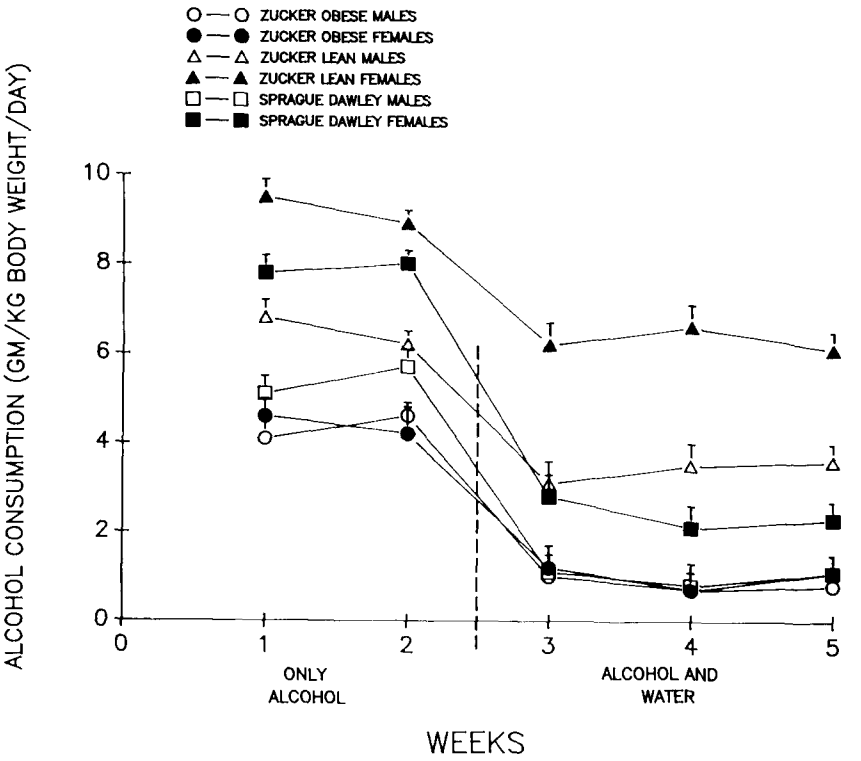

FIG. 3. Daily alcohol consumption, expressed as grams of alcohol consumed (absolute) per kg body weight, of the different groups and sexes of rats during the first 5 weeks of the study. *Differences between sexes were only apparent for the ZL rats during the whole period and for the SD rats during the forced alcohol consumption period (weeks 1, 2). There were no sex differences in alcohol consumption in the ZO rats.

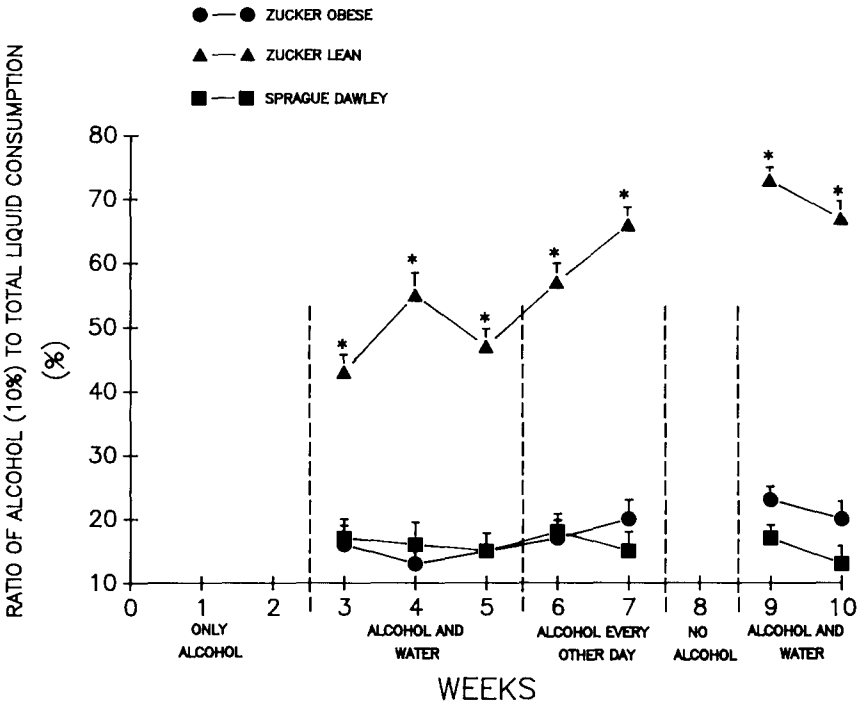

FIG. 4. Ratio between alcohol solution (10%) : total liquid consumption of the different groups during the experimental period. *The alcohol to liquid ratio used by Zucker lean (ZL) rats was significantly ($p < 0.05$) greater than either ZO or SD rats.

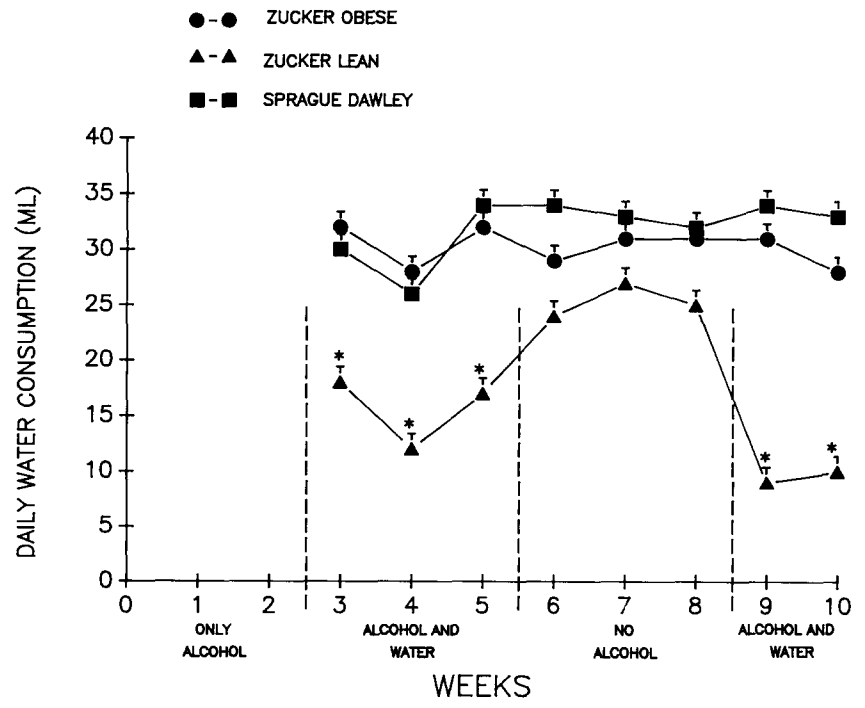

FIG. 5. Daily water consumption of the different groups of rats during the experimental period. *Zucker lean (ZL) rats consumed significantly ($p < 0.05$) less water than either ZO or SD rats.

Following the two weeks of alternate-day alcohol deprivation, rats were deprived completely of alcohol for one week (week 8); then they were provided with both the 10% alcohol solution and water ad lib. There was no significant difference among the different groups or sexes in the amount of alcohol consumed during the first hour after one week of alcohol deprivation. However, the ZL rats consumed significantly more ($p < .05$) alcohol (threefold) than the ZO or SD rats (Fig. 1). It was also apparent that alcohol consumption during the first week after alcohol deprivation (week 9) was higher in general than consumption for the preceeding week (week 7). This rate of increase was significantly higher ($p < 0.5$) for the ZL groups than for either the ZO or SD rats. It was also observed that during the second week of this phase there was a gradual decrease in alcohol consumption by all animals. This significant decline ($p < .05$) of daily alcohol consumption in weeks 9–10 is shown in Fig. 2.

When alcohol consumption data were converted to g of alcohol consumed (absolute) per kg body weight (Fig. 3), differences between sexes were apparent only in the ZL group. The ZL females consumed consistently more alcohol per kg body weight than the ZL males. In almost all cases, a similar pattern of alcohol consumption was observed (i.e., ZL rats consumed more alcohol than either the ZO or SD rats; Fig. 3).

Figure 4 represents the data of alcohol consumption expressed as percent of total liquid intake. There were no sex differences in the three groups studied. However, ZL rats consumed a significantly higher ($p < .05$) alcohol : liquid ratio than the other two groups (Fig. 4). Water consumption data (Fig. 5) indicated an inverse relationship with the alcohol consumption data. Differences between the groups indicated that the ZO and SD rats consumed significantly more ($p < .05$) water when both alcohol and water were offered. Differences between sexes for all groups were not significant.

Changes in the body weights of the three groups during the experiment are presented in Fig. 6. It can be seen from that figure that during forced alcohol intake period (weeks 1–2) animals gained weight, except for the ZO females, who lost weight by the end of the second week. When the animals were offered both the water and alcohol solutions simultaneously (week 3 to week 4), the ZO males and females had a sudden increase in body weight (Fig. 6).

DISCUSSION

The results obtained in this experiment clearly show that a single gene can alter alcohol preference in animals. The data show that ZL rats consistently and significantly consumed more alcohol than their ZO littermates. During the three-week period (weeks 3–5) when the animals received both water and alcohol ad lib, the ZL rats consumed significantly more alco-

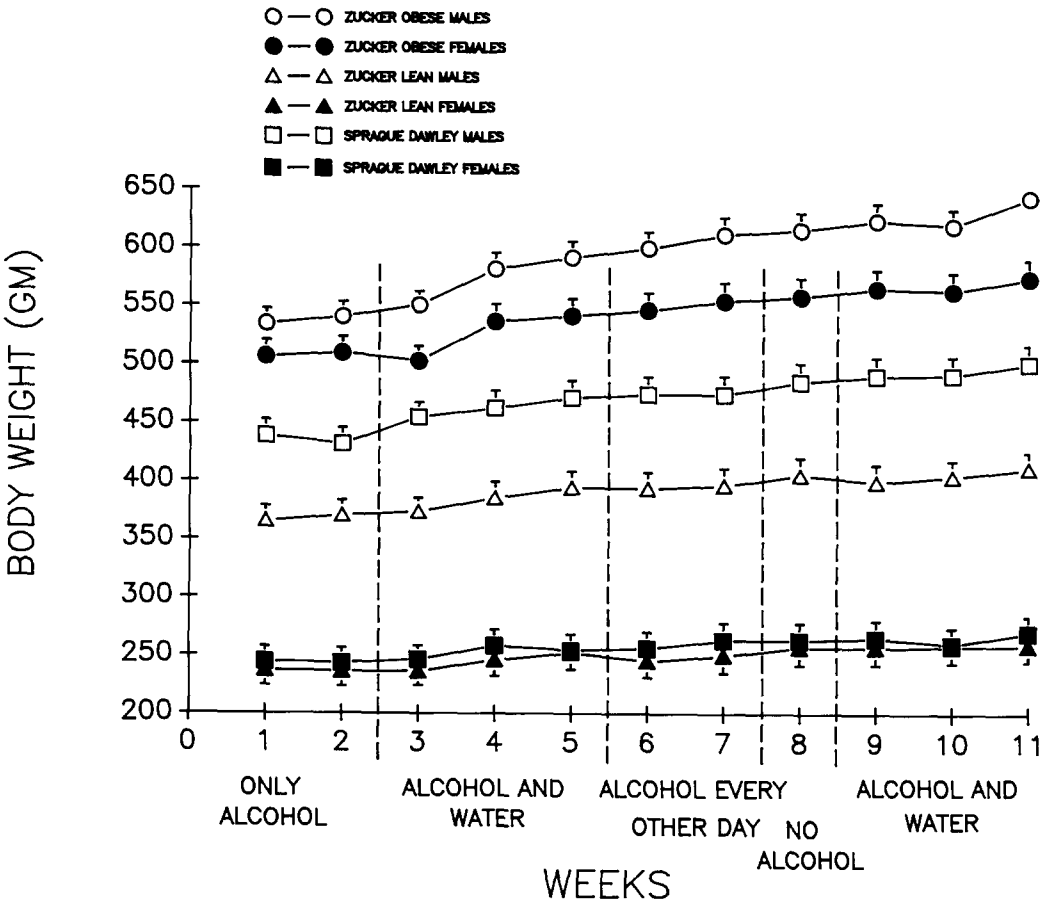

FIG. 6. Weekly body weights (at the beginning of each week) of the different groups of rats over the experimental period.

hol than the other two groups. This coincided with water intake reduction and was reflected in the alcohol : total liquid ratio.

Alcohol deprivation on alternate days caused an increase in alcohol consumption for the ZL rats. This was true for both alcohol solution consumption and the alcohol : total liquid consumption ratio. The effect of alternate-day alcohol deprivation on alcohol intake on the other two groups was not noticeable, which demonstrates the intensity of alcohol preference in ZL rats. Earlier results indicated that rat lines genetically selected for alcohol preference consumed more alcohol than those selected for low alcohol intake when they were deprived of alcohol on alternate days (16).

Following two weeks of alternate-day alcohol deprivation, rats were deprived of alcohol completely for a week (week 8) and then offered both alcohol and water ad lib (weeks 9-10). In this paradigm, the ZL rats again increased their alcohol consumption to the highest level of the study. The level of ZL rats' alcohol consumption closely approached the level of their forced alcohol consumption during the first two weeks of the study. Their alcohol : liquid ratio reached 73% during the first week after alcohol deprivation. The significant increase in alcohol consumption by ZL rats after one week of alcohol deprivation indicates that this group of rats, unlike the other two groups, preferred the alcohol solution (11) and that their preference was enhanced by alcohol deprivation.

In the present study, the ZL females consumed significantly more alcohol (g alcohol per kg body weight) than ZL males (Fig. 3). A similar trend was also observed in Long-Evans rats (9). This sex difference was not observed in ZO rats because of the similarity in the male and female body weights. Sex differences in alcohol consumption per kg body weight were also observed for the SD rats when they were forced to drink the alcohol for the first two weeks of the study (Fig. 3). Differences in alcohol consumption between ZL and ZO (g/kg body weight/day) rats might have been exaggerated by the large differences in their body weights (Figs. 3 and 6).

The data obtained in the present study showed that ZL rats had a high preference ratio for alcohol consumption (up to 73%), compared to both ZO and SD rats. This may indicate that the fa/fa gene, a single gene, or the associated obesity in the ZO rats is the main reason for the difference in alcohol preference between ZO and ZL rats.

Obesity may be associated with taste aversion to certain types of tastes (5), including the taste of alcohol solutions. Indeed, ZO rats did not drink as much alcohol solution when they had a choice. It is of interest to note that overeating in rats might be associated with the animals' preference for a specific food taste (15). In a recent experiment, it was found that taste plays a major role in the development of obesity in Zucker rats (7). It was also reported that saccharin induced significantly more release of insulin in ZO rats than in ZL rats (7).

Because they were littermates, the main difference between the ZL and ZO rats is only one gene. The ZO rats are homozygotic (fa/fa); most ZL rats are heterozygotic (Fa/fa), and the rest are Fa/Fa. The present results indicate that the fa/fa gene directly or indirectly influences alcohol preference. The nature of the influence, however, was somewhat different from what might be expected. Numerous parameters are known to be abnormal in the ZO rats (17,19). These include higher levels of serum insulin (18), dysfunctions in the pituitary-adrenal axis (1) and the pituitary-thyroid axis (2), depressed serum thyroxine levels (12), and a lower metabolic rate (3). Furthermore, various brain regions of the ZO rats have recently been found to contain significantly higher levels of the cholinergic enzymes (6). Some of these differences in the ZO rats might affect alcohol drinking.

The alcohol drinking by ZO rats, however, was not different from that of the SD animals. Instead, the ZL rats were found to be different from both ZO and SD animals. It is possible that the abnormalities present in the obese rats do suppress their drinking, while the intake by SD rats is suppressed to a similar degree by some other factors. Alternatively, the Fa/fa heterozygosity of the majority of ZL rats may have elevated both their mean level of alcohol drinking and their responsiveness to alcohol deprivation.

In conclusion, the data presented in this experiment support the theory that genetic factors contribute to alcohol preference. The data also indicate a connection between alcohol drinking and the fa/fa gene responsible for the obesity in the ZO rats. The observed connection does not support the widely held belief that overeating is associated with alcohol abuse. Instead, the ZL rats, the littermates of the obese animals, were found to be high alcohol consumers.

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