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Rat strain-specific differences in alcohol intake following patterned feeding of a palatable diet†

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Abstract: 29.5 million people aged 12 and older met the diagnostic criteria for alcohol use disorder (AUD) in the United States in 2021, which presents a significant social and economic burden to modern society. Impaired nutritional status has been frequently documented in patients with AUDs and could contribute to escalated alcohol consumption and behavioral impairments commonly observed in AUD. Interestingly, increased highly palatable food intake during recovery has been reported in patients with AUD, suggesting the importance of understanding the relationship between palatable food and problematic alcohol drinking. We have previously shown that patterned feeding of a palatable diet attenuated alcohol drinking in Long Evans rats. The present study evaluated the impact of patterned feeding on high and low alcohol drinking. Individually housed male high drinking (P), moderate drinking (Long Evans), and low drinking (Wistar) rats received intermittent access (24 hours, Tuesdays, and Thursdays) to a nutritionally complete high fat diet (Int-HFD) or standard chow (controls). Normal chow and water were available ad libitum to all groups of rats. Intermittent HFD access induced a feeding pattern in which the Int-HFD group of rats escalated their caloric intake on Tuesdays and Thursdays. Two weeks of Int-HFD pre-exposure preceded any alcohol access, after which all rats were given unsweetened alcohol (20% v/v) in their home cages via a two-bottle choice paradigm of voluntary alcohol drinking. Alcohol was available for 24 hours on chow only days (Mondays, Wednesdays, Fridays) while Int-HFD feeding continued. Long Evans rats receiving the Int-HFD displayed a significant ~50% reduction in alcohol drinking when compared to controls. The Int-HFD group of P rats also reduced their alcohol intake significantly ($p<0.05$), ~20%, in comparison to respective controls. Interestingly, alcohol drinking in Wistar rats was not affected ($p>0.05$) by intermittent HFD exposure. These data highlight rat strain specific differences in alcohol intake following patterned feeding of a palatable diet and identify Long Evans rats as an ideal model to evaluate impact of palatable diet on alcohol drinking.

Keywords: Wistar; Long Evans; P rat; Alcohol Use Disorder; Alcohol Drinking; High Fat Diet; Palatable Diet

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1. Introduction

Alcohol use disorder (AUD) is a debilitating disorder which significantly impacts an individual's health and ability to function and has extensive economic impacts. An estimated 140,000 people die annually of alcohol-related causes [1] and the life expectancy of someone with AUD has been shown to be reduced by as much as 28 years when compared to healthy individuals [2]. Additionally, when measured in disability-adjusted life-years, alcohol misuse contributes significantly to years of life lost due to improper health or disability [3]. In 2010, the cost of alcohol misuse in the United States (US) totaled

$249 billion and $191.1 billion of this financial burden was attributed to binge drinking [4].

Impaired nutritional status is frequently reported in patients with AUD along with emotional and physiological abnormalities. The cause of nutritional deficiency in this population is multifaceted, impacted by reduced nutritional intake, altered nutrient absorption, and changes in nutrient metabolism [5-7]. Particularly, deficiencies in vitamins and minerals have been widely reported in people with AUD [8]. For example, Vitamin B12 and C levels are negatively impacted by excessive alcohol consumption and are associated with cognitive dysfunction [9-10]. Vitamin D is another essential nutrient affected by chronic alcohol intake and these deficiencies have been implicated in increased negative affect [11]. Importantly, unlike other substances of abuse, alcohol contains calories and when the calories from alcohol replace those typically obtained from a healthy diet, nutritional status is negatively affected. In individuals with severe AUD more than 30% of daily caloric intake could be derived from alcohol alone, which negatively affects dietary carbohydrate, fat, and protein intake [12]. Together, a compromised nutritional status as a result of chronic alcohol consumption could impair health and could contribute to AUD and related pathologies.

Interestingly, increased preference for palatable diets (PDs), or food high in sugar [13–15] and carbohydrates [14], have been observed in people with AUD during recovery, suggesting potentially protective effects of increased PD intake in people with AUD [13-15]. Furthermore, Alcoholics Anonymous also suggest sweet-tasting foods consumption to curb alcohol cravings [16]. Several preclinical studies from our laboratory and others have also evaluated the impact of PD consumption on alcohol drinking [17-19]. For example, our lab has previously demonstrated reduced alcohol deprivation effect following intermittent high-fat diet (Int-HFD) access [17] and attenuated alcohol drinking following two weeks of Int-HFD pre-exposure [18] in Long Evans rats. The objective of the present study was to compare the impact of two weeks of Int-HFD pre-exposure on subsequent alcohol drinking in lower-drinking Wistar rats, moderately drinking Long Evans rats, and higher-drinking P rats.

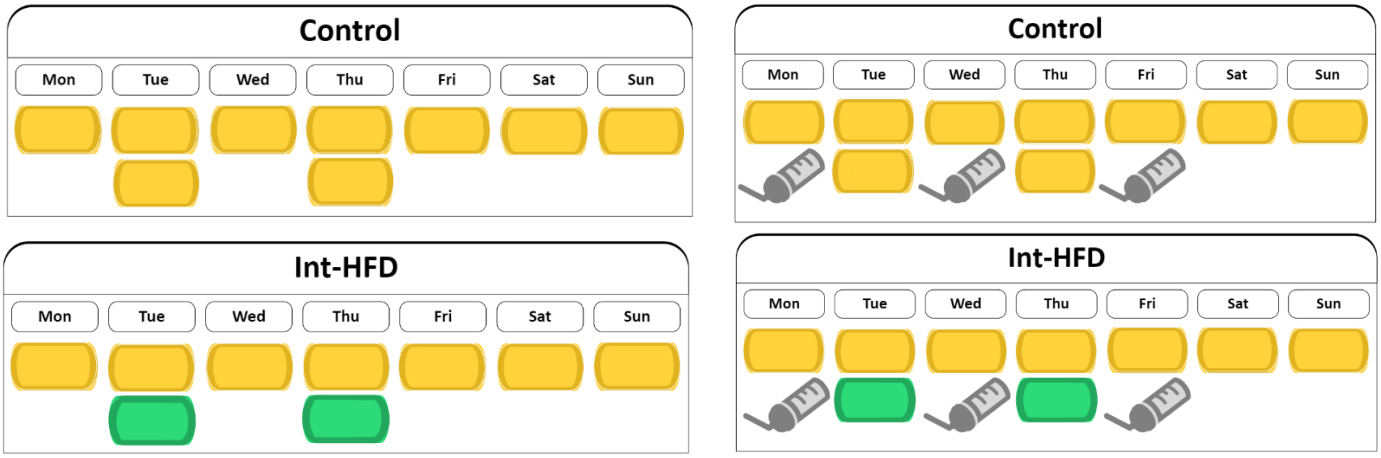
2. Materials and Methods

2.1. Animals

Male Wistar (RccHan®: WIST, Envigo RMS, Inc, Indianapolis, IN), Long Evans (HsdBlue: LE, Envigo RMS, Inc, Indianapolis, IN) and alcohol-preferring (P) rats (Indiana University) were used. The vivarium was controlled for temperature (~70F) and humidity (~60%) with a 12-hour reverse light-dark cycle (lights on from 1:00 AM to 1:00 PM). On arrival, animals were handled before any experimental manipulation or baseline data (body weight, food intake, water intake) were collected.

2.2. Diet and Alcohol

All animals had ad libitum access to standard rodent chow (Tekland-Envigo Diets #2020X, 3.1 kcal/g with 16% calories from fat and 60% calories from carbohydrates) and tap water. The experimental group was given intermittent access to a high-fat diet (HFD; Research Diets #D03082706, 4.5 kcal/g with 40% calories from fat, 46% calories from carbohydrates, and 7.9% calories from sugar) in addition to standard chow. 190 proof alcohol was purchased from Greenfield Global, MI and the desired 20% v/v was prepared at least one day in advance every week. Voluntary alcohol consumption was measured in home cages with a 2-bottle choice paradigm and the position of the alcohol and water bottles were switched daily to minimize conditioning effects. Food, alcohol, and water were provided ~2 hours into the dark cycle, and intake was measured manually after 24 hours of access.



A **B**

Figure 1. Schematic representation of patterned feeding and alcohol access schedules. Green boxes represent high-fat diet (HFD) access, yellow boxes represent chow access, and the grey bottles represent alcohol access. **(A)** Intermittent high-fat diet (Int-HFD) rats were given 2 weeks of intermittent 24-hour HFD access (Tuesday and Thursday) while controls received additional chow. **(B)** Following HFD pre-exposure, rats received alcohol access on chow only days (Monday, Wednesday, Friday) simultaneous to Int-HFD feeding.

2.3. Procedure

Male rats (n=6) matched for body weight, food intake, and water intake were randomly divided into control and Int-HFD groups. To evaluate the effect of Int-HFD feeding on subsequent alcohol intake, Int-HFD rats were given 24-hour, intermittent (Tuesday, Thursday) access to the HFD for two weeks prior to alcohol exposure (see Figure 1A). Following Int-HFD pre-exposure, alcohol consumption was measured on chow-only days (Monday, Wednesday, Friday) while intermittent HFD feeding continued (see Figure 1B).

3. Results and Discussion

Consistent with our previously published data, all groups of Int-HFD rats displayed caloric overconsumption on HFD access days. Long Evans rats displayed a gradual increase in alcohol drinking over time, an effect significantly (50%) reduced in the Int-HFD group of rats. When evaluated under identical conditions, alcohol intake in Int-HFD P rats was also significantly (~20%) reduced compared to chow controls, whereas no effect of Int-HFD on alcohol intake was evident in Wistar rats. Together, intermittent access to HFD differentially impacted alcohol intake in low, moderate, and high drinking rats with greater effects seen in moderate drinking Long Evans rats. The present study emphasizes strain specific differences in the effect of an intermittent HFD on subsequent alcohol intake.

It is important to note that several critical factors could impact alcohol drinking following dietary manipulations. For example, significant differences in the behavioral effects of a high-sugar diet and a high-fat diet have been previously identified [20], emphasizing dietary content as a factor requiring further evaluation. Furthermore, the length of PD exposure may have an impact on alcohol drinking. In the present study, all the experimental parameters were kept constant between rat strains and there was no significant change in body weight observed in any Int-HFD rats when compared to respective controls throughout the experiment. Interestingly, a previous study reported attenuated alcohol intake in Wistar rats following 3-4 weeks of junk-food diet (averaging 42% fat, 52% carbohydrates, 6% protein) access. In contrast to the present study, junk-food

diet feeding induced an obesity phenotype [19], emphasizing the potential impact of PD access periods duration.

In alignment with previous published reports, Wistar rats in the current study displayed a low initiation of alcohol drinking and minimal escalation of intake over time. On the other hand, P rats displayed a high initiation of alcohol drinking, maintained throughout the testing period. Interestingly, pervious studies comparing blood alcohol levels (BALs) during an intermittent alcohol access paradigm reported higher BALs in Long Evans rats when compared to Wistar and P-rats. Even with lower alcohol intake, the amount of ethanol consumption required by P rats and Wistar rats to reach the same pharmacologically relevant blood ethanol content (BEC) was greater than that required by Long Evans rats [21-23]. We have previously reported involvement of central mechanisms in mediating the effects of an intermittent HFD on alcohol drinking, as selective alterations in the neurotransmitter receptor expression in brain reward circuitry were observed in Int-HFD Long Evans rats when compared to chow controls [18]. Therefore, the low alcohol drinking levels and likely lower BALs of the Wistar rats in the present study could explain lack of intermittent HFD access effects on alcohol drinking, a contention needing further evaluation.

4. Conclusions

In conclusion, there was no observed effect of intermittent HFD access on alcohol intake in the low-drinking Wistar rats. Int-HFD P rats displayed significantly attenuated alcohol intake (20%) when compared to the chow controls, however the effect was smaller than that observed in Int-HFD Long Evans rats (50%). These results emphasize rat strain specific differences in the effect of an Int-HFD on subsequent alcohol intake and warrant future investigation. Nevertheless, this study identifies Long Evans rats as a potentially ideal model for evaluating central mechanisms of diet-induced effects on alcohol intake.

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Informed Consent Statement:

Data Availability Statement:

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Conflicts of Interest: Authors declare no conflict of interest.

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