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Dissociation between diurnal cycles in locomotor activity, feeding behavior and hepatic PERIOD2 expression in chronic alcohol-fed mice

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ABSTRACT

Chronic alcohol consumption contributes to fatty liver disease. Our studies revealed that the hepatic circadian clock is disturbed in alcohol-induced hepatic steatosis, and effects of chronic alcohol administration upon the clock itself may contribute to steatosis. We extended these findings to explore the effects of chronic alcohol treatment on daily feeding and locomotor activity patterns. Mice were chronically pair-fed *ad libitum* for 4 weeks using the Lieber-DeCarli liquid diet, with calorie-controlled liquid and standard chow diets as control groups. Locomotor activity, feeding activity, and real-time bioluminescence recording of PERIOD2::LUCIFERASE expression in tissue explants were measured. Mice on liquid control and chow diets exhibited normal profiles of locomotor activity, with a ratio of 22:78% day/night activity and a peak during early night. This pattern was dramatically altered in alcohol-fed mice, marked by a 49:51% ratio and the absence of a distinct peak. While chow-diet fed mice had a normal 24:76% ratio of feeding activity, with a peak in the early night, this pattern was dramatically altered in both liquid-diet groups: mice had a 43:57% ratio, and an absence of a distinct peak. Temporal differences were also observed between the two liquid-diet groups during late day. Cosinor analysis revealed a ~4–h and ~6–h shift in the alcohol-fed group feeding and locomotor activity rhythms, respectively. Analysis of hepatic PER2 expression revealed that the molecular clock in alcohol-fed and control liquid-diet mice was shifted by ~11 h and ~6 h, respectively. No differences were observed in suprachiasmatic nucleus explants, suggesting that changes in circadian phase in the liver were generated independently from the central clock. These results suggest that chronic alcohol consumption and a liquid diet can differentially modulate the daily rhythmicity of locomotor and feeding behaviors, aspects that might contribute to disturbances in the circadian timing system and development of hepatic steatosis.

Introduction

The endogenous circadian timing system, or clock, regulates behavior and physiologic activity in concert with the light–dark cycle and ensures that daily rhythms in metabolism are temporally coordinated with rest, activity, and feeding cycles. In mammals, the master circadian oscillator resides in the hypothalamic suprachiasmatic nucleus (SCN), which synchronizes and phase-resets the cell autonomous clocks in peripheral tissue through humoral signals, body temperature cycles, and feeding-related cues (Mohawk, Green, & Takahashi, 2012). A cell-autonomous circadian clock controls local hepatic physiology by driving rhythmic gene and protein expression, and these in turn impact time-of-day specific regulation of many processes including lipid and glucose metabolism, and bile acid (BA) synthesis (Bailey, Udoh, & Young, 2014; Marcheva et al., 2013).

Hepatic steatosis, the accumulation of triglyceride (TG) droplets in the hepatocytes, is the earliest response of the liver to excessive alcohol consumption (Sozio, Liangpunsakul, & Crabb, 2010). Alcohol metabolism reduces the nicotinamide adenine dinucleotide/

Abbreviations: BAC, blood alcohol concentration; CAF, chronic alcohol feeding; CG, center of gravity; ED, ethanol-containing diet; LCD, liquid control diet; RD, regular chow diet; LD, light:dark; ZT, Zeitgeber time; SCN, suprachiasmatic nucleus; BA, bile acid; TG, total cholesterol; FA, fatty acid; PIR, passive infrared; PER2::LUC, PERIOD2::LUCIFERASE.

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reduced nicotinamide adenine dinucleotide (NAD/NADH) ratio, resulting in interrupted mitochondrial fatty acid (FA) β oxidation and thus leading to hepatic steatosis (Sozio et al., 2010). FA, cholesterol, and BA synthesis fluctuate over the diurnal cycle with a circadian periodicity, as does the expression of genes associated with their regulation (Zhou, Ross, Pywell, Liangpunsakul, & Duffield, 2014). Feeding mice with Lieber-DeCarli alcohol-containing diet for 4 weeks results in both hepatic steatosis as well as disturbances to the hepatic circadian clock (Zhou et al., 2014).

There is emerging evidence that the time of day at which meals are consumed influences behavioral and physiological systems. Reverse feeding of nocturnally active rodents during the light phase of the light:dark (LD) cycle results in increased weight gain (Arble, Bass, Laposky, Vitaterna, & Turek, 2009). Human studies suggest that shift workers who have altered meal times are at a higher risk of developing obesity and metabolic syndrome (Wang, Armstrong, Cairns, Key, & Travis, 2011). Human participants who confined their energy intake to the early day lost more weight than those who consumed a similar diet later in the day (Garaulet et al., 2013).

Given the drawbacks of the ethanol-in-drinking-water procedure, which does not result in alteration in hepatic pathology as observed in humans with heavy drinking, the Lieber-DeCarli ethanol liquid diet was developed in response to the need to develop an animal model with an alcohol consumption of clinical relevance (Lieber, DeCarli, & Sorrell, 1989; Matson et al., 2013). The liquid diet results in high ethanol intake, high blood alcohol level, and evidence of hepatic steatosis after 24 days of access (DeCarli & Lieber, 1967; Lieber & DeCarli, 1970), and has become the standard method of inducing liver steatosis in rodents. Three standardized basic formulas are used widely: 1) an all-purpose (35% fat kcal) Lieber-DeCarli diet, which is appropriate for most experiment applications, 2) a high-protein formula diet (25% protein kcal) useful in pregnancy and lactation, and 3) a low-fat formula diet (12% fat kcal), which is intended to minimize the hepatic changes by fat consumption (Lieber et al., 1989; Matson et al., 2013).

Our previous study using the low-fat Lieber-DeCarli diet (Zhou et al., 2014) revealed that chronic alcohol feeding (CAF) interferes with the normal operation of the hepatic circadian clock, leading to loss of temporal coordination of liver-associated metabolic function at both the gene expression and physiological levels. Furthermore, another group reported differences in the diurnal expression of clock genes in the liver of chronically alcohol-fed mice (Filiano et al., 2013), a finding broadly consistent with our studies. Furthermore, it is known that adaptation of peripheral clocks is dependent on the time of feeding, meal frequency pattern, and metabolic state (Kuroda et al., 2012). However, currently there are few studies systematically characterizing the feeding and locomotor activity patterns in mice chronically fed with the Lieber-DeCarli diet. As such, the data presented here build upon our previous studies by elucidating the effects of chronic alcohol treatment on daily feeding and locomotor activity patterns, and on the circadian phase of the liver clock in CAF mice.

Materials and methods

Animals and diets

C57BL/6 male mice from Jackson Laboratory (Bar Harbor, ME) and PER2::LUCIFERASE mice (PER2::LUC; C57BL/6J background) generated from in-house breeding at University of Notre Dame (UND) (Yoo et al., 2004) were housed individually in a room with controlled temperature (20–22 °C) and humidity (55–65%). Mice were first entrained to a 12:12 light:dark (LD) cycle for 4 weeks and maintained on a regular chow diet. The experimental design consisted of three dietary groups: 1) liquid control diet (LCD) (Calories – 10:18:72; by % calculation as fat: protein: carbohydrate; Lieber-DeCarli liquid diet, Dyets Inc., Bethlehem, PA), 2) ethanol-containing diet (ED) (identical to the LCD, except that ethanol was added to account for 27.5% of total calories and the caloric equivalent of carbohydrate [maltose-dextrin] removed), and 3) regular chow diet (RD) (Calories – 22:23:55; Teklad Global diet 2919) (Mathew et al., 2013; Zhou et al., 2014). Alcohol was introduced gradually into the diet during the first 5 days of feeding starting from 9% alcohol (of the total calories) for 2 days, 18% for the next 3 days, and then finally 27.5% until the end of the experiment (Liangpunsakul et al., 2012; Zhou et al., 2014). Ethanol-containing diet or LCD were available *ad libitum* and dispensed using a standard calibrated 50–mL liquid diet feeding tube (Bio-Serv, Flemington, NJ) attached to the end of the cage (Bertola, Mathews, Ki, Wang, & Gao, 2013). A separate water source was also provided using a standard water bottle. Fresh food was prepared daily by homogenization in a blender and routinely changed between Zeitgeber Time (ZT) 2 and ZT3 (ZT0 = lights on [morning], ZT12 = lights off [evening]). Ethanol-containing diet or LCD was provided in equal volumes (25 mL each) at the time of food change. Feeding activity was measured (see details below) during the 2-h span (1 h before and 1 h after) after the food was changed. Mice were weighed weekly and food consumption (volume) was measured every day for 4 weeks. Experiments were approved by the UND IACUC and performed in accordance with NIH Guidelines for the Care and Use of Laboratory Animals.

Feeding and locomotor activity recording

Mice (aged 9–12 weeks) were housed individually with a 12:12 LD cycle, and chronically pair-fed *ad libitum* for 4 weeks using ED (not including the first 5 days of alcohol introduction), or with LCD or RD as control groups. Feeding activity was assessed by either video monitoring (SONY HDR-PJ790 Digital HD video camera recorder) and behavior scoring as duration (seconds) feeding at the liquid feeder (ED and LCD; see Supplemental Video Sv1), or by visits to the food hopper in specialized cages (RD) and as previously described (Mathew et al., 2013). The Smart Cutter program (SWREG, Inc., Minnetonka, MN, USA) was used for video editing. Locomotor activity was assessed by passive infrared (PIR) motion detection (Mathew et al., 2013). RD feeding activity and three groups of PIR activity were monitored using *Clocklab* hardware and software (Actimetrics, Wilmette, IL). For RD feeding and PIR locomotor activity, individual animal data were selected for analysis: Minute-to-minute data were recorded over 5 consecutive days and occurring within 25 days of the onset of experimentation. Liquid diet (ED and LCD) video footage and PIR activity were recorded for individual animals for 24 h. All data from each mouse were averaged into hourly bins and converted to percentage values, with the maximum values as 100% for each mouse.

Biochemical measurements

Whole blood was collected at 4–h intervals (ZT0, 4, 8, 12, 16, and 20, $n = 1$ [ZT0–12]; $n = 2$ at ZT16 and ZT20) by an intracardiac puncture and clotted in Microtainer tubes (BD, Franklin Lakes, NJ, USA) for 45 min, and serum was extracted. Blood alcohol levels were determined using the Alcohol Reagent Set (Pointe Scientific, Inc., Canton, Michigan) (Filiano et al., 2013).

Real-time luminescence recording and analysis

PER2::LUC mice were pair-fed with ED, LCD, or RD for 4 weeks. Mice were sacrificed at ZT4 or ZT8. Explant cultures were prepared as previously described (van der Veen, Shao, Xi, Li, & Duffield, 2012; Zhou et al., 2014). Tissues were placed in a light–tight box at 36 °C,