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Alcohol Suppresses Cardiovascular Diurnal Variations in Male Normotensive Rats: Role of Reduced PER2 Expression and CYP2E1 Hyperactivity in the Heart

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Abstract

Background and Aims: The molecular mechanism of the adverse effects of ethanol on diurnal cardiovascular regulation remains unknown. In separate studies, the cardiac circadian rhythm protein period-2 (PER2) confers cardioprotection and, in other organs, PER2 interaction with the ethanol-metabolizing enzyme CYP2E1 underlies, via heme oxygenase-1 (HO-1) upregulation, tissue injury/dysfunction. Here, we hypothesized that suppressed PER2 expression and elevated CYP2E1/HO-1 levels in the heart underlie the disrupted diurnal cardiovascular rhythm/function in alcohol-fed normotensive rats.

Methods: In ethanol-fed (5%, w/v; 8 weeks) or isocaloric liquid diet-fed male rats, diurnal changes in blood pressure (BP), heart rate (HR), HR vagal variability index, root mean square of successive beat-to-beat differences in beat-interval duration (rMSSD), and cardiac function were measured by radiotelemetry and echocardiography followed by *ex vivo* molecular studies.

Results: Radiotelemetry findings showed ethanol-evoked reductions in BP (during the dark cycle), rMSSD (during both cycles), and in diurnal differences in BP and rMSSD. Echocardiography findings revealed significant ($p < 0.05$) reductions in ejection fraction and fractional shortening (weeks 4–6) in the absence of cardiac remodeling (collagen content). Hearts of ethanol-fed rats exhibited higher ($p < 0.05$) CYP2E1 activity (50%) and HO-1 expression (63%), along with reduction ($p < 0.05$) in PER2 levels (29%), compared with the hearts of isocaloric diet-fed control rats.

Conclusions: Our novel findings implicate upregulations of CYP2E1/HO-1 and downregulation of the circadian rhythm cardioprotective protein PER2, in the heart, in the chronic deleterious diurnal cardiovascular effects of alcohol in male rats.

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Authorship contributions

Research design: Abdel-Rahman; conducted experiments & performed data analysis: Katary; wrote or contributed to the writing of the manuscript: Katary, Abdel-Rahman.

Conflict of interest

None.

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Keywords

blood pressure; cardiac CYP2E1; cardiac PER2; circadian rhythm; ethanol; heart rate; HO-1

Introduction

Reported findings have demonstrated the complexity of the favorable vs. detrimental cardiovascular effects of alcohol (Krenz & Korthuis, 2012; Perissinotto et al., 2010). The net result is determined by the balance between multifaceted cardiac as well as hypertensive and vasodilator effects of alcohol. The hypertensive effect of ethanol is mediated by sympathoexcitation (Brunner et al., 2017), cardiac baroreflex impairment (Crestani et al., 2014), and stimulation of the renin-angiotensin system (Marchi, Muniz, & Tirapelli, 2014) and cortisol (Adinoff, Ruether, Krebaum, Iranmanesh, & Williams, 2003). On the other hand, the hypotensive effect of ethanol is attributed to its enhancement of vascular nitric oxide release (Kaewphaleuk, Watanapa, & Panich, 2019), stimulation of adenosine receptors (Rekik, El-Mas, Mustafa, & Abdel-Rahman, 2002), direct vasodilator action (Narkiewicz, Cooley, & Somers, 2000), and myocardial depression (Ma et al., 2010). Therefore, the hypotensive effect of alcohol could result from reduced cardiac function or independently via vascular relaxation (El-Mas & Abdel-Rahman, 2019; Piano, 2017).

The diurnal blood pressure (BP) rhythm, characterized by a higher daytime BP that dips at night in humans (Douma & Gumz, 2018), is reversed in rodents due to their nocturnal nature (Cooper, Carter, March, & Woolard, 2019). Evidence suggests that smaller dips in night-time BP and attenuated heart rate variability (e.g., rMSSD) are independent risk factors for cardiovascular diseases (Aksit, Gursul, Aydin, Samsa, & Ozcelik, 2017; Yano & Kario, 2012). Further, evidence from human and rodent studies suggests that induction (Davis, Voigt, Shaikh, Forsyth, & Keshavarzian, 2018) or inhibition (Filiano et al., 2013) of an organ (liver) circadian machinery such as period 2 (PER2), via the overexpressed ethanol-metabolizing enzyme CYP2E1, contributes to the adverse effects of alcohol on liver physiology. However, much less attention has been devoted to studying the effects of alcohol on diurnal cardiovascular regulation and cardiac PER2-CYP2E1 interaction, despite an association between alcohol consumption with increases in morning BP (Auer & Rodondi, 2009) and cardiomyopathy (Maisch, 2016).

While many local circadian rhythm proteins modulate organ physiology, the key circadian clock protein PER2 (Yeom, Lee, Shin, Park, & Jung, 2018) plays a pivotal role in BP diurnal control (Vukolic et al., 2010) and in regulating vascular function (Viswambharan et al., 2007). Therefore, it is possible that the ethanol-evoked disruption of the diurnal BP profile in humans (Aguilera et al., 1999; Stiffler, Suter, & Vetter, 1999) is mediated via disrupted cardiovascular PER2 expression. This premise is partly supported by alcohol-evoked attenuation of the diurnal differences in BP and rMSSD in male rats, although these findings might be confounded by hypertension (El-Mas & Abdel-Rahman, 2000, 2005a). Currently, there are no preclinical studies on the potential role of cardiac PER2 and its interaction with CYP2E1 or other alcohol-metabolizing enzymes in the adverse effects of alcohol on diurnal cardiovascular regulation.

Studying the link between PER2, ethanol-metabolizing enzymes, and other enzymes such as heme oxygenase-1 (HO-1), which regulate tissue PER2 level (Damulewicz, Loboda, Jozkowicz, Dulak, & Pyza, 2017), is warranted for the following reasons. First, tissue CYP2E1 induction of PER2 is implicated in organ (liver and intestine) pathology (Davis et al., 2018). Second, CYP2E1 and other ethanol-metabolizing enzymes (ADH and catalase) catalyze ethanol oxidation to acetaldehyde, which mediates cardiac dysfunction via other cardiotoxic molecules such as malondialdehyde (MDA) (El-Mas & Abdel-Rahman, 2019; Yao & Abdel-Rahman, 2017). Third, cardiac CYP2E1 mediates cardiomyopathy (Maisch, 2016), while PER2 serves a cardioprotective role (Bonney et al., 2013). Therefore, cardiac CYP2E1 hyperactivity might lead to reduced PER2 expression in the heart, contrary to other organs.

The present study was designed to first determine whether chronic ethanol consumption adversely influences the diurnal BP and HR variability and cardiac function in male normotensive rats. Next, we measured circadian clock-regulated PER2 and its interactive enzymes, the alcohol-metabolizing enzymes and HO-1, along with oxidative stress in the heart, to understand the mechanisms of the adverse effects of alcohol consumption on cardiovascular diurnal function.

Materials and methods

Animals

Male Sprague-Dawley rats (300–325 g, 9–10 weeks old) were purchased from Charles River (Raleigh, North Carolina, United States). The rats were housed individually in standard plastic cages in the animal facility at East Carolina University, and were maintained on a 12/12-hour light/dark cycle with the lights off at 7:00 PM. Room temperature was maintained at 22 ± 1 °C. All surgical procedures were approved by the East Carolina University Institutional Animal Care and Use Committee and were consistent with the Guide for the Care and Use of Laboratory Animals as governed by the U.S. National Institutes of Health (2015).

Experimental design

One week after telemetry transmitter implantation, rats were housed individually and randomly divided into two groups (n = 7 each; divided into four and three rats with and without telemetry implants, respectively). Ethanol was provided, during the first acclimation week (2.5%, w/v) and during weeks 2–8 (5%, w/v) in a phytoestrogen-free liquid diet (Alcohol rodent liquid diet, LD 102A, Test diet, Lab supply Inc.; Fort Worth, Texas, United States). The control group received isocaloric liquid diet (Rodent liquid diet, 530A, Test diet, Lab supply Inc.; Fort Worth, Texas) according to the pair-feeding paradigm. Fresh diets were prepared every day, and rats were maintained on ethanol or control diet for 8 weeks. Blood was withdrawn biweekly via tail vein for measurements of blood ethanol, MDA, and protein carbonyl content. In the morning (9:00 AM) of the last day of the study, rats from the ethanol-fed and control groups were paired to undergo anesthesia with ketamine and xylazine (90/10 mg/kg) for blood and tissue collection for conducting the biochemical studies. There were no differences in the daily intakes of liquid diet with alcohol or

isocaloric diet or the growth in body weights between the rats with or without the telemetry implants throughout the study (Supplementary Figure 1).

Telemetry diurnal studies

A radiotelemetry system (Data Sciences Int.; St. Paul, Minnesota, United States) was used in this study as described in our previous studies (El-Mas & Abdel-Rahman, 2003, 2004), with modification. Briefly, under inhaled isoflurane 2% anesthesia, a 1.5-cm incision was made in the left thigh to uncover the femoral vessels (n = 4 per group). An HD-S10 transmitter was subcutaneously implanted with its catheter tip inserted into a femoral artery, and advanced into the abdominal aorta. After surgery, postoperative analgesia (meloxicam, 5 mg/kg) was administered orally for 3 days. Individual rat cages were placed on radio receivers, and all data were collected using a dedicated data acquisition system (Ponemah P3P, Data Sciences Int.; St. Paul, Minnesota). BP waveforms were sampled at a rate of 1000 Hz for 10 seconds every 10 minutes. Mean blood pressure, heart rate, and motor activity were averaged weekly during light (7:00 AM to 7:00 PM) and dark (7:00 PM to 7:00 AM) periods to study the diurnal BP and cardiovascular responses in ethanol- and isocaloric diet-fed rats throughout the 8 weeks of the experiment.

Time-domain analyses

Heart rate variability was determined by computing the root mean square of successive beat-to-beat differences in beat-interval durations (rMSSD), an index of cardiac vagal input (Sgoifo et al., 1997; Stein, Bosner, Kleiger, & Conger, 1994), as in our previous studies (El-Mas & Abdel-Rahman, 2005a, 2005b).

Measurement of plasma ethanol concentration

Blood ethanol concentration was measured as detailed in our previous study (Yao & Abdel-Rahman, 2016, 2017). The absorbance was distinguished at 340 nm with a microplate reader (Tencan Group Ltd.; Männedorf, Switzerland).

Echocardiography studies

Echocardiography was performed before starting ethanol feeding, and biweekly at 9:00 AM thereafter, following a standard protocol (Visual Sonics Vevo 3100 Imaging System, FujiFilm, and VevoLab Software v.2.1.0; Toronto, Ontario, Canada) as described in our recent study (Leffler & Abdel-Rahman, 2019). Briefly, under inhalation (2% isoflurane) anesthesia, rats were transferred to an imaging platform, and the M-mode as well as B-mode images were applied to measure cardiac structure and function, left ventricle (LV) mass, ejection fraction, and fractional shortening. In addition, the color Doppler mode was used to measure an aortic velocity-time interval of the blood flow. It is noteworthy that echocardiography procedure did not affect daily food intake.

Measurement of ADH, ALDH2, and catalase activities

Cardiac alcohol dehydrogenase (ADH), aldehyde dehydrogenase 2 (ALDH2), and catalase (CAT) activities were measured by commercial kits for ADH (Sigma-Aldrich; St. Louis, Missouri, United States), and for ALDH2 and CAT (Abcam; Ann Arbor, Michigan, United

States). We followed the protocols provided by the manufacturer and used in our studies (Ibrahim, Fan, & Abdel-Rahman, 2014; Yao & Abdel-Rahman, 2016, 2017).

Measurement of cytochrome P450 2E1 (CYP2E1) activity

CYP2E1 activity was measured in heart tissue homogenate by the technique of oxidation of p-nitrophenol (PNP) to p-nitrocatechol by CYP2E1 in the presence of NADPH as reported (Yao & Abdel-Rahman, 2020; Zhang et al., 2011). Briefly, 200 μL of reaction medium containing 500 μg protein, 100 mM potassium-phosphate buffer, 2 mM PNP, and 1 mM NADPH was incubated at 37 °C for 60 minutes, then terminated by 30 μL of 20% trichloroacetic acid. After centrifugation (10,000 rpm for 10 minutes; approximately 16,000 g), 200 μL of the supernatant was mixed with 10 μL of 10 M NaOH in a 96-well plate. Finally, using a microplate reader (Tencan Group Ltd.; Männedorf, Switzerland), the optical density was read at 546 nm.

Oxidative stress markers

Direct measurement of ROS levels with high accuracy and precision is difficult due to their stability issues (Katerji, Filippova, & Duerksen-Hughes, 2019). Therefore, markers of oxidative modification of the lipids and proteins such as MDA and protein carbonyl levels provide more reliable measures of oxidative stress (Katerji et al., 2019). TBARS Assay Kit (Cayman Chemical; Ann Arbor, Michigan) for measuring myocardial and plasma MDA levels and a protein carbonyl assay kit (Cayman Chemical; Ann Arbor, Michigan) were used in accordance with the manufacturer's protocol.

Western blot

We followed the western blot protocols described in our study (Yao & Abdel-Rahman, 2020). The supernatant (50 μg of protein) of the homogenized LV tissue was separated in a 12% gel electrophoresis (Novex Tis-Glycine gel, Life Technologies; South San Francisco, California, United States), transferred to nitrocellulose membranes, and then blocked using Odyssey blocking buffer (LI-COR Biosciences; Lincoln, Nebraska, United States) and incubated with primary antibodies: rabbit monoclonal anti-PER2 (1:200, ab179813 Abcam; Ann Arbor, Michigan), rabbit polyclonal anti-heme oxygenase 1 (1:200, ab13243), and mouse anti-beta actin (1:1000, ab8226, Abcam; Ann Arbor, Michigan). Thereafter, the membranes were incubated with a secondary antibody (IRDye680-conjugated goat anti-mouse and IRDye800-conjugated goat anti-rabbit, 1:1000, LI-COR Biosciences; Lincoln, Nebraska), and the protein bands were detected and quantified by Odyssey Infrared Imager (LI-COR Biosciences; Lincoln, Nebraska).

Collagen hybridizing peptides (CHP) staining

As described (Hwang et al., 2017), frozen left ventricles were embedded in optimal cutting temperature (OCT) compound and sectioned (20 μm) with a cryostat (HM 505E; Microm International GmbH; Waldorf, Germany). Then, the OCT compound was removed by rinsing with PBS. After blocking with 5% goat serum, heat-activated solutions of single-strand CHPs were added to the tissue sections. Then, the sections were heated for 5 minutes using an 80 °C water bath to dissociate the trimeric peptides, followed by immediate incubation in

an ice/water bath and incubated in a humidity chamber at 4 °C overnight. The fluorescence was visualized by a Zeiss LSM 510 microscope (Zeiss; Oberkochen, Germany) and quantified using Image J Software (National Institutes of Health).

Statistical analysis

Values are presented as mean ± SEM. The time-course data were analyzed by repeated-measures two-way ANOVA followed by a *post hoc* test (Bonferroni), while unpaired *t* tests were performed when comparing two groups and the areas under the curve (AUC), which measured the cumulative effects using Prism version 5 (GraphPad Software, Inc.; La Jolla, California). A p value <0.05 was considered as statistically significant.

Results

Chronic ethanol consumption reduced the gain in body weight

While daily liquid diet intake was similar in ethanol- and isocaloric diet-fed control rats (Figure 1A), body weight was lower ($p < 0.05$) in ethanol-fed rats toward the end of the study (Figure 1B). In agreement with our previous findings (El-Mas & Abdel-Rahman, 2000), blood ethanol concentration (BEC) peaked during the study (week 4) and started to decline thereafter in ethanol-fed rats (Figure 1C), despite steady ethanol intake (Figure 1D).

Ethanol dampened circadian variations in blood pressure and rMSSD without affecting the activity

Control isocaloric diet-fed rats exhibited normal diurnal BP rhythm observed as higher ($p < 0.05$) mean arterial pressure (MAP) (Figure 2A, C) and heart rate (HR) (Figure 2D, F) and lower ($p < 0.05$) rMSSD (Figure 2G, I) during the dark cycle, compared to the light period. Ethanol reduced ($p < 0.05$) the dark cycle MAP (Figure 2B, C) and the rMSSD during both cycle phases (Figure 2H, I), along with dampening ($p < 0.05$) the diurnal MAP (Figure 2C) and rMSSD (Figure 2I) variations, but had no effect on HR (Figure 2F). The dark-cycle activity counts similarly increased ($p < 0.05$) from similar light-cycle activity levels in both ethanol-fed and control pair-fed (Figure 2J, K) rats, indicating no effect of ethanol, compared with isocaloric diet, on rat activity counts (Figure 2L).

Ethanol caused myocardial dysfunction

Echocardiographic findings revealed ethanol-evoked reductions ($p < 0.05$) in ejection fraction (Figure 3A, B) and fractional shorting (Figure 3C, D), which were resolved by the end of the study. Ethanol had no effect on an aortic velocity-time interval (Figure 4A, B) or left ventricular (LV) mass, assessed by echocardiography (Figure 4C, D), and by the post mortem heart weight/tibia length ratio (Figure 4E). The latter findings were corroborated by the lack of ethanol effect on LV collagen levels when compared to isocaloric liquid diet-fed rats (Figure 5).

Ethanol downregulated PER2 and upregulated CYP2E1 and HO-1 in the heart

The hearts of ethanol-fed rats exhibited reduced ($p < 0.05$) PER2 level (Figure 6A) along with higher ($p < 0.05$) CYP2E1 activity (Figure 6B) and HO-1 expression (Figure 6C),

compared to isocaloric diet-fed rats. Notably, the expression level of the inducible enzyme HO-1 reflects its activity (Nassar, Li, Strat, & Abdel-Rahman, 2011). The activities of the other ethanol-metabolizing enzymes (ADH, catalase, and ALDH2) were similar in the hearts of ethanol-fed and isocaloric diet-fed rats (Figure 6D–F).

Ethanol elevated plasma and myocardial oxidative stress

Ethanol increased ($p < 0.05$) plasma levels of MDA (Figure 7A) and protein carbonyl (Figure 7B) starting at week 4, and continuing until the conclusion of the study. Similarly, at the conclusion of the study (week 8), the hearts of ethanol-fed rats exhibited higher ($p < 0.05$) MDA (Figure 7C) and protein carbonyl (Figure 7D) levels, indicating increased lipid peroxidation and protein oxidation levels, compared to pair-fed control rats.

Discussion

Mounting evidence implicates tissue PER2 and CYP2E1 interactions in organ physiology and pathology. This is the first preclinical study to discern the chronic effects of ethanol on cardiac CYP2E1-PER2 interaction in relation to the diurnal cardiovascular regulation and function. Compared with isocaloric liquid diet-fed rats, the following are the most important telemetry, echocardiographic, and biochemical findings in ethanol-fed rats (5% w/v; 8 weeks): i) suppressed dark cycle BP and HR variability (rMSSD) and dampened diurnal variations in both variables in the absence of any change in rat activity counts; ii) reduced cardiac PER2 level; and iii) higher cardiac CYP2E1 activity, HO-1 expression, and oxidative stress. Collectively, the findings suggest that an imbalance, within the heart, between the cardioprotective circadian rhythm protein PER2 (suppressed) and its interrelated pro-oxidant enzymes CYP2E1 and HO-1 (increased) explains, at least partly, the ethanol-evoked suppressions of diurnal cardiovascular regulation and heart rate variability. Importantly, these latter anomalies are well-established risk factors for cardiovascular morbidity and mortality.

The alcohol regimen (5% w/v in liquid diet) used in the current study and in our previous studies (El-Mas & Abdel-Rahman, 2007) lead to blood ethanol concentrations comparable to those achieved following moderate to heavy alcohol consumption in humans (Piano, 2017). It was important to establish the *in vivo* effects of ethanol on diurnal cardiovascular regulation and function in the model system used in the present study for two reasons. First, ethanol was added to a phytoestrogen-free liquid diet (controls received the isocaloric version of the same diet) to circumvent the possible impact of these estrogenic compounds on the study outcomes. Second, we utilized the new generation telemetry system and expanded our studies to include parallel echocardiography measurements. In agreement with reported findings including our findings (El-Mas & Abdel-Rahman, 2005a; Giles, 2006; Redon, 2004; Shea, Hilton, Hu, & Scheer, 2011), isocaloric liquid diet-fed control rats exhibited normal diurnal differences in BP and HR – higher during the dark cycle (active), compared to the light cycle (Figure 2). Chronic ethanol consumption dampened the BP diurnal variability mainly by reducing the dark cycle BP, which agrees with experimental and clinical findings (El-Mas & Abdel-Rahman, 2005a, 2005b; Stiffler et al., 1999). It is

noteworthy that similar activity counts in the ethanol and control groups (Figure 2) ruled out reduced activity as a possible cause for the lower dark cycle BP in ethanol-fed rats.

It is also important to discuss the clinical ramifications of ethanol-evoked dampening of the diurnal variations in rMSSD (Figure 2G–I). These findings are consistent with alcohol-evoked suppression of heart rate variability due to reduced vagal and increased sympathetic dominance in humans (Spaak et al., 2010). It is also notable that the observed adverse diurnal cardiovascular effects persisted after the resolution of the ethanol-evoked reductions in ejection fraction (EF) and fractional shortening (FS) at week 8 (Figure 3), and in the absence of LV hypertrophy (Figure 4C–E) or LV remodeling (Figure 5). The lack of LV hypertrophy, assessed by echocardiography and by heart weight/tibia length, and the lack of remodeling, assessed by cardiac collagen content, in ethanol-fed rats agree with similar reported findings (Ninh et al., 2018; Steiner, Pruznak, Navaratnarajah, & Lang, 2015).

The following reasons may explain the recovery of ejection fraction (EF) and fractional shortening (FS) by the end of the study despite steady alcohol intake. First, there was a decline in blood alcohol level from its peak at week 4 (Figure 1C), despite consistent daily alcohol intake, which agrees with our previous findings (El-Mas & Abdel-Rahman, 2000). Second, a concurrent compensatory sympathoexcitation counterbalances the chronic alcohol-evoked cardiac dysfunction in male rats (El-Mas & Abdel-Rahman, 2000). It is noteworthy that sympathoexcitation-evoked cardiac autonomic dysfunction (Ibrahim et al., 2014), which likely explains the cardiac autonomic dysfunction in alcohol-fed rats (Figure 2G–I), is implicated in long-term adverse cardiovascular effects (Gilligan, Chan, Sbarouni, Nihoyannopoulos, & Oakley, 1993; Spaak et al., 2010).

After establishing the adverse effects of ethanol on diurnal BP and rMSSD in our model system, we studied the role of cardiac PER2 and its interaction with two cardiac enzymes, HO-1 and CYP2E1, in these clinically relevant effects. Mounting evidence supports critical roles for tissue circadian rhythm proteins in organ physiology and pathology, including the cardiovascular system (Crnko, Du Pré, Sluijter, & Van Laake, 2019; Morris, Yang, & Scheer, 2012). Specifically, plasma PER2 levels in alcoholic men (Huang et al., 2010) and in the liver are reduced, along with liver injury, in ethanol-fed mice (Filiano et al., 2013) although other researchers implicate higher PER2 in liver pathology (Davis et al., 2018). Importantly, a cardiovascular protective role for PER2 is generally accepted (Bonney et al., 2013; Eckle et al., 2012; Vukolic et al., 2010) because its gene mutation reduces the diurnal differences in BP (Leu et al., 2015; Vukolic et al., 2010) and in vascular endothelial function (Viswambharan et al., 2007). Therefore, our novel clinically relevant findings suggest that the downregulated cardiac PER2 expression (Figure 6) contributes, at least partly, to the dampened diurnal BP control and cardiac dysfunction caused by ethanol (Figure 2). We accounted for the possibility that variability in the time of tissue collection might influence the generated *ex vivo* biochemical data on the studied circadian rhythm-regulated proteins. This was accomplished by collecting blood and tissues from paired ethanol-fed and isocaloric liquid diet-fed rats in the morning.

Here, we discuss the inverse relationship between tissue oxidative stress and PER2 level (Tong et al., 2015) and a controversial role for HO-1 as inhibitor (Kosuru et al., 2018) or

mediator (Fouda & Abdel-Rahman, 2017) of oxidative stress to explain our molecular and *in vivo* findings. Our novel finding of ethanol-evoked reduction in cardiac PER2 is consistent with a cardioprotective role for cardiac PER2 (Bonney et al., 2013; Eckle et al., 2012; Vukolic et al., 2010). Further, we present the first evidence of CYP2E1 hyperactivity (Figure 6B) and HO-1 overexpression (Figure 6C) in the hearts of ethanol-fed rats. The CYP2E1 hyperactivity (Figure 6) likely contributed to the adverse cardiac effects of ethanol via generation of higher levels of its cardiotoxic metabolite, acetaldehyde (Yao & Abdel-Rahman, 2017), as well as via CYP2E1-mediated adverse cardiac effects (Maisch, 2016). Therefore, the upregulations of the pro-oxidant enzymes, CYP2E1 and HO-1, likely contribute to the oxidative stress, observed as higher LV MDA and carbonyl levels (Figure 7), and the PER2 reduction (Figure 6). This premise is supported by an inverse relationship between HO-1 and PER2 in the other tissues (Damulewicz et al., 2017), and by the contribution of oxidative stress to hepatotoxicity (Zeng et al., 2018) and myocardial dysfunction (Zhang et al., 2013) caused by alcohol. This interpretation gains further credence from mechanistic findings that implicate CYP2E1 induction of HO-1 in tissue oxidative stress and injury in the kidney (Wang, Shah, Liu, & Baliga, 2014) and liver (García-Suástegui et al., 2017).

Finally, it is important to comment on PER2 role as antioxidant protein. For example, PER2 plays a role in oxidative stress production and redox system because *per2* mutant mice exhibit upregulated oxidative stress (Hardeland, Coto-Montes, & Poeggeler, 2003) via Akt (Wang et al., 2008). Interestingly, Akt signaling mediates ethanol-evoked cardiac oxidative stress (El-Mas, Fan, & Abdel-Rahman, 2009). Notably, as a co-activator of peroxisome proliferator-activated receptor alpha (Chappuis et al., 2013), PER2 is linked to cardiac superoxide dismutase expression and improvement of cardiac function (Guellich et al., 2007). Collectively, these findings support the premise that the downregulated cardiac PER2 contributes, at least partly, to cardiac oxidative stress in the alcohol-fed rats in the present study. Future studies are warranted to discern the role of major redox enzymes such as superoxide dismutase, glutathione peroxidase, or glutathione reductase in our model system.

In conclusion, the present findings yield new insights in support of the premise that upregulations of CYP2E1 activity and HO-1 level along with downregulation of PER2 level in the heart contribute, at least partly, to the deleterious diurnal cardiovascular effects of alcohol in male rats.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Abbreviations

ADH	alcohol dehydrogenase

ALDH2	aldehyde dehydrogenase 2
BP	blood pressure
CYP2E1	cytochrome P2E1
HO-1	heme oxygenase-1
HR	heart rate
MAP	mean arterial pressure
MDA	malondialdehyde
PER2	period 2
rMSSD	root mean square of successive beat-to-beat differences in beat-interval duration

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Highlights

- Alcohol suppresses dark cycle BP, HR variability, and dampened diurnal variations.
- Alcohol-fed rats exhibited reduced cardiac PER2 expression and CYP2E1/HO-1 hyperactivities.
- Imbalance between the cardioprotective PER2 (reduced) and pro-oxidant enzymes, CYP2E1 and HO-1, contribute to the adverse effects of alcohol on cardiovascular diurnal function.

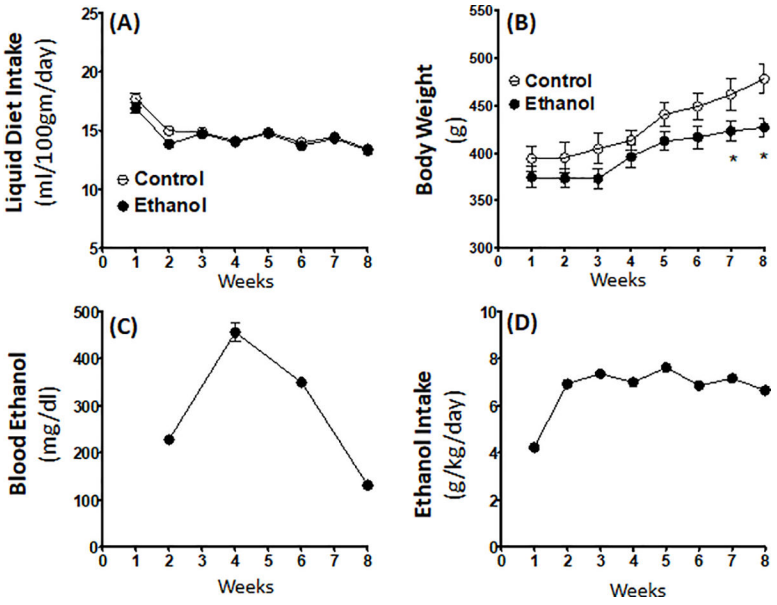

Figure 1.

Similar liquid diet intake in ethanol-fed (2.5% during week 1, and 5% w/v thereafter) and pair-fed control rats (**A**) resulted in lower body weight in ethanol-fed rats toward the end of the study (**B**). Blood ethanol concentrations peaked at week 4 and started to decline thereafter in ethanol-fed rats (**C**) despite a steady ethanol intake throughout the study (**D**). Values are mean ± SEM (n = 7). *$p < 0.05$ versus corresponding control liquid diet pair-fed rats.

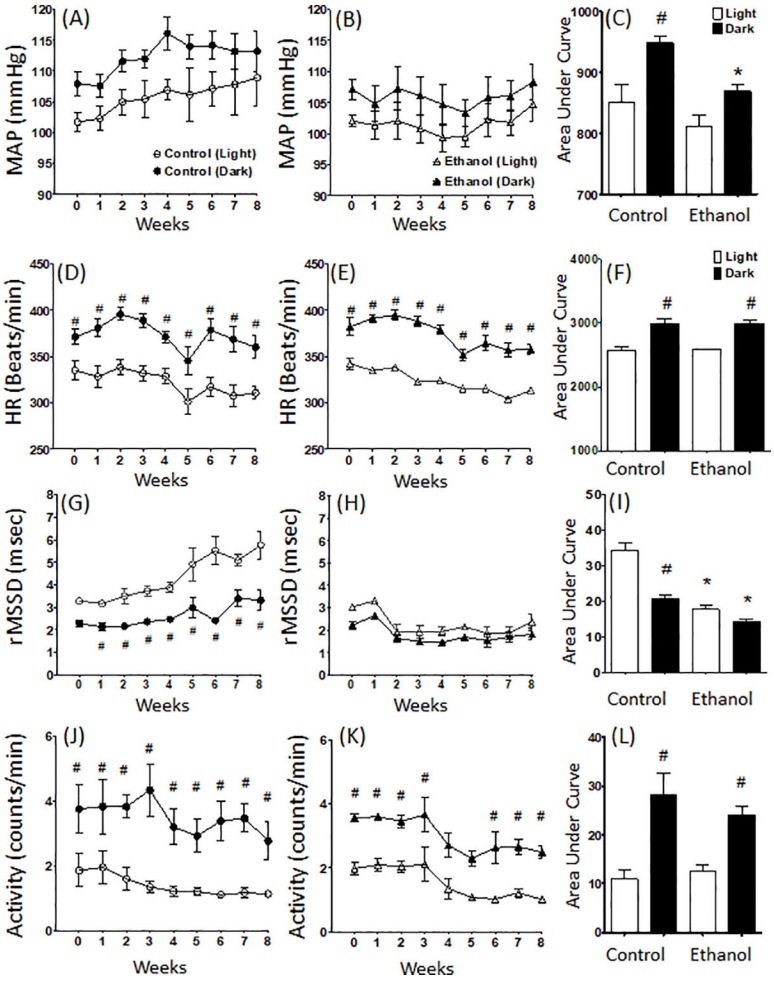

Figure 2.

Diurnal (light/dark) profile of mean arterial blood pressure (MAP) (**A, B**), heart rate (HR) (**D, E**), root mean square of successive beat-to-beat differences in beat-interval durations (rMSSD) (**G, H**), and motor activity (**J, K**) in control pair-fed and ethanol-fed rats. Area under the curve data show that ethanol (5% w/v) reduced MAP during the dark cycle (**C**), reduced rMSSD during both cycle phases (**I**), and had no effect on HR (**F**) or activity counts (**L**). Values are mean ± SEM (n = 4). *$p < 0.05$ vs. corresponding control pair-fed rats. #$p < 0.05$ vs. corresponding light cycle values.

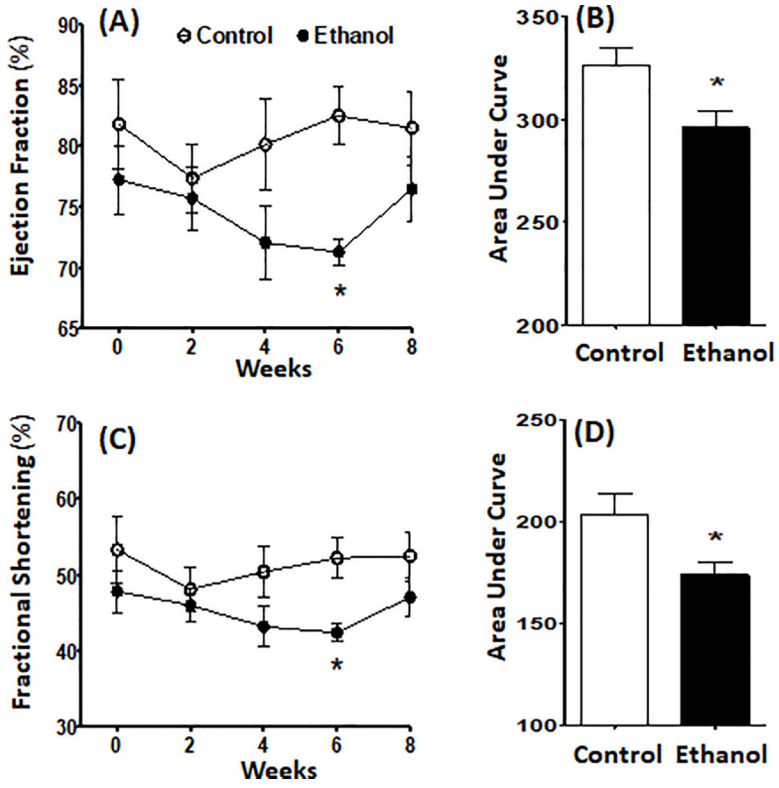

Figure 3.

Time-course echocardiographic findings showing ethanol-evoked reductions in two cardiac contractility indices, ejection fraction (**A**), and fractional shortening (**C**) during the first 6 weeks and aggregate (area under the curve) reductions in both variables (**B, D**) during the study. Values are mean ± SEM (n = 7). *$p < 0.05$ versus corresponding control pair-fed rats.

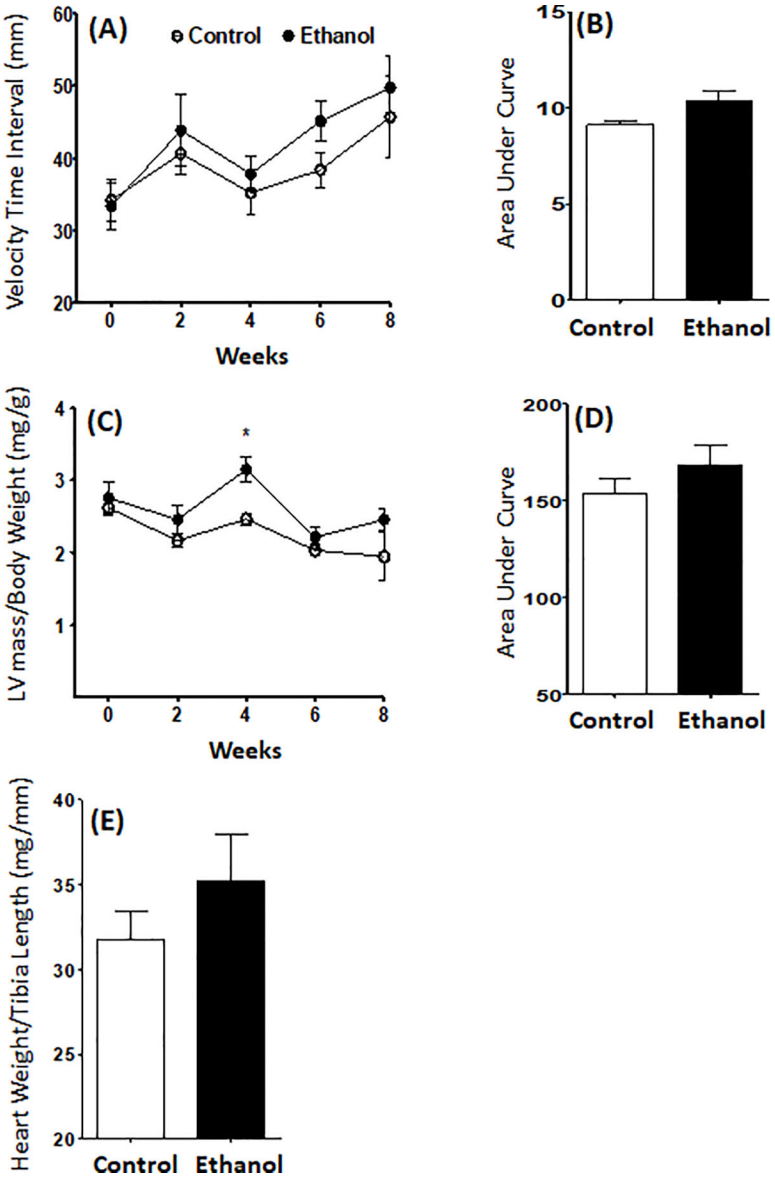

Figure 4.

Time-course (**A & C**) and area under the curve (**B & D**) echocardiographic findings showing no effect of ethanol on aortic velocity-time interval (**A & B**) and normalized left ventricular (LV) mass to body weight, respectively. The latter was corroborated with a lack of difference in normalized heart weight to tibia length ratios in ethanol-fed and liquid diet-fed control rats (**E**). Values are mean ± SEM (n = 7). *$p < 0.05$ versus corresponding control pair-fed rats.

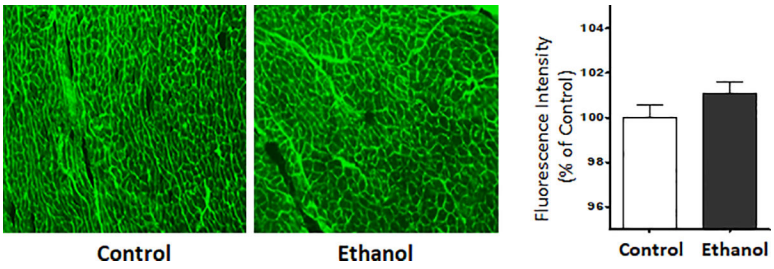

Figure 5.

Representative confocal fluorescence images (4 sections per rat) of myocardial tissue stained with collagen hybridizing peptides (CHP) and group data show no difference between ethanol-fed and control pair-fed rats. Values are mean ± SEM (n = 7). Scale bar: 50 μm.

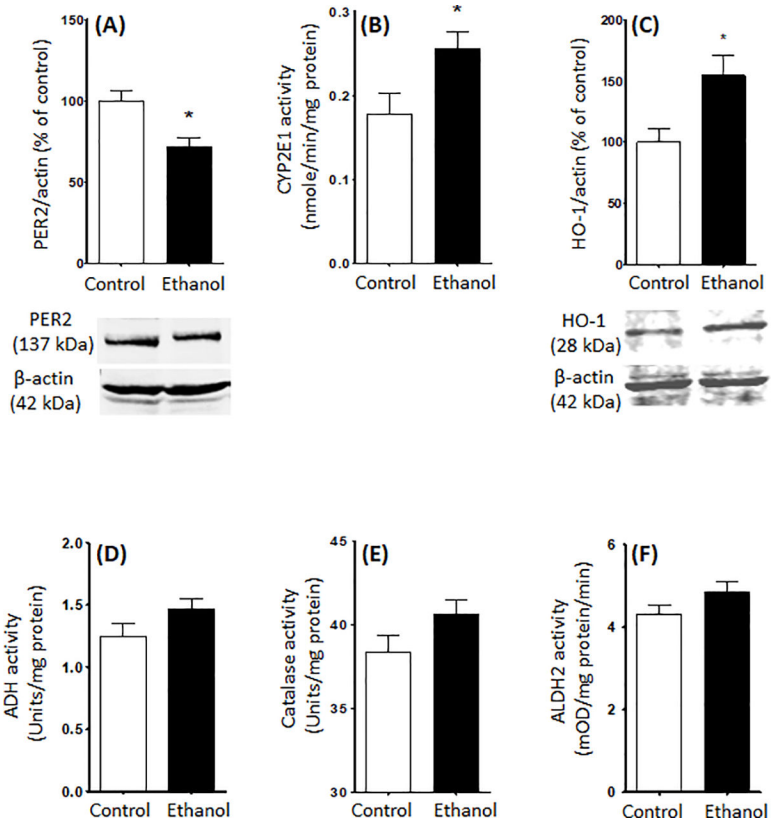

Figure 6.

Chronic ethanol (5% w/v) feeding reduced cardiac PER2 expression (**A**) while enhancing both CYP2E1 activity (**B**) and heme oxygenase (HO1) expression (**C**), compared to corresponding levels in liquid diet-fed control rats. The activities of the other ethanol-metabolizing enzymes ADH (**D**), catalase (**E**), and ALDH2 (**F**) were similar in the hearts of ethanol-fed and control pair-fed rats. Values are mean ± SEM (n = 7). *$p < 0.05$ versus corresponding control values.

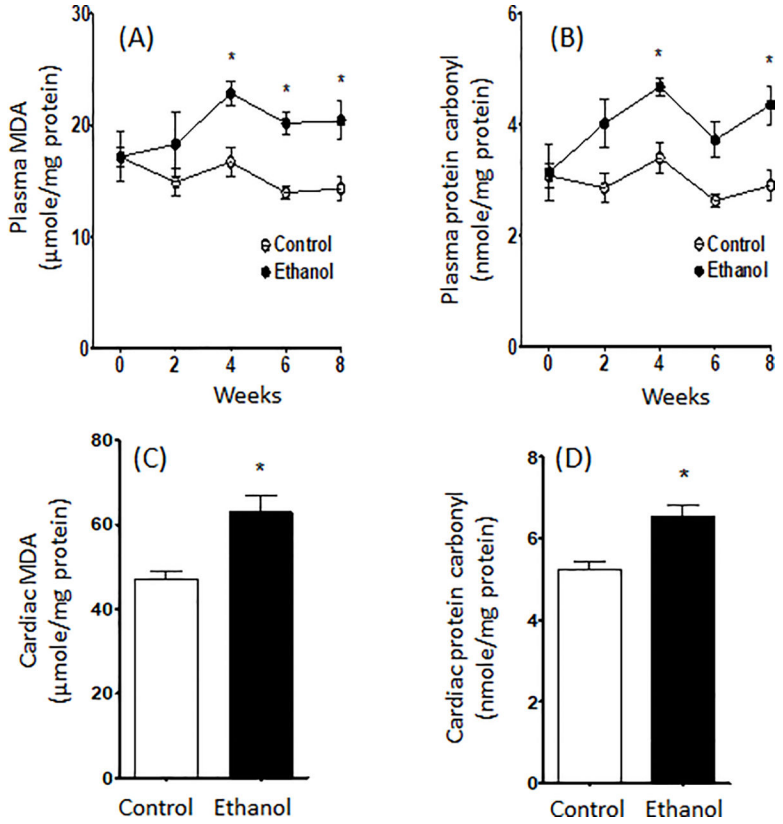

Figure 7.

Chronic ethanol (5% w/v) feeding increased plasma MDA (**A**) and protein carbonyl levels (**B**) starting at week 4 as well as cardiac levels of both oxidative stress markers (**C, D**) at the end of the study, compared to corresponding levels in liquid diet-fed control rats. Values are mean ± SEM (n = 7). *$p < 0.05$ versus corresponding control values.

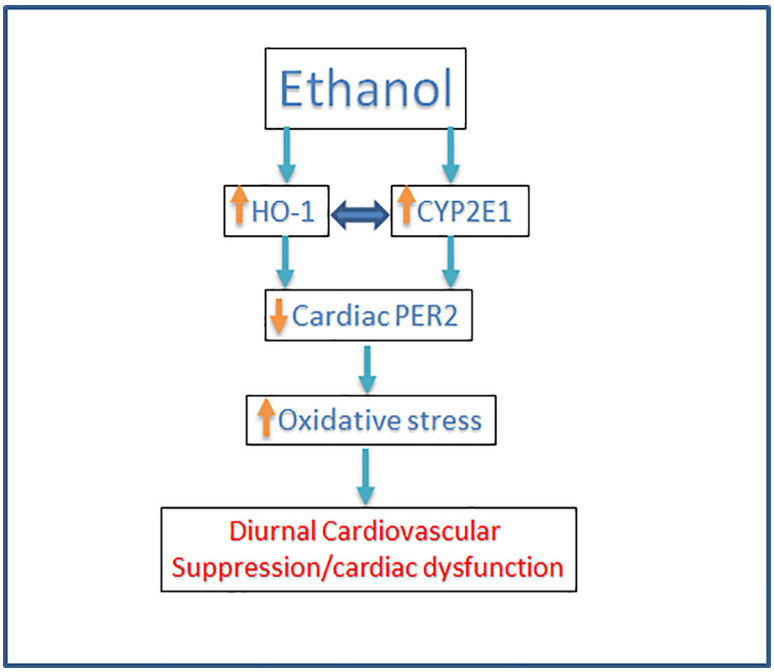

Figure 8.

Schematic presentation of the suggested roles of the inverse relationships between reduced PER2 and elevations in CYP2E1 activity and HO-1 expression, in the heart, in the ethanol-evoked detrimental effects on diurnal cardiovascular rhythmicity and cardiac function in male normotensive rats (see discussion for details).