



Published in final edited form as:

Adv Exp Med Biol. 2018 ; 1071: 129–136. doi:10.1007/978-3-319-91137-3_16.

Chronic heart failure abolishes circadian rhythms in resting and chemoreflex breathing

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Abstract

Physiological systems often display 24hr rhythms that vary with the light/dark cycle. Disruption of circadian physiological rhythms have been linked to the progression of various cardiovascular diseases, and advances in the understanding of these rhythms have led to novel interventions and improved clinical outcomes. Although respiratory function has been known to vary between the light and dark periods, circadian rhythms in breathing have been understudied in clinical conditions. In the current study, we have begun to assess light/dark variations in respiration in chronic heart failure (CHF), a condition associated with abnormal resting and chemoreflex breathing as well as exercise intolerance. CHF was induced using coronary artery ligation and verified using echocardiography. Sham animals underwent a thoracotomy without coronary artery ligation. Tidal volume, respiratory frequency, and minute ventilation were all determined by whole body plethysmography under resting conditions and in response to chemoreflex challenges during the light and dark periods. Light/dark differences in voluntary exercise were assessed using a running wheel. The sham control group showed light/dark differences in resting and chemoreflex breathing, as well as arterial pressure, and these effects were eliminated in the CHF group. Both groups completed more rotations on the running wheel during the dark period compared to during the light period. The data suggest that CHF disrupts cardiovascular and respiratory circadian rhythms.

Keywords

circadian rhythm; chronic heart failure; chronobiology; plethysmography

1.1 Introduction

Many cellular and physiological processes display cyclic patterns with the same periodicity as the 24hr light/dark cycle (Partch et al. 2014), and these circadian rhythms appear to be disrupted in a variety of clinical conditions (Durgan and Young 2010; Ayala et al. 2013). Among the circadian rhythms in physiology, rhythmic changes in cardiovascular function have perhaps been most studied. Research into circadian rhythms in cardiovascular physiology and pathophysiology has led to recent clinical efforts to utilize knowledge of these rhythms in treating cardiovascular diseases (Tsimakouridze et al. 2015), and these

efforts have been shown to reduce adverse cardiac remodeling, buffer the morning surge in blood pressure, and reduce the number of adverse cardiovascular events (Tsimakouridze et al. 2015). While advances in chronobiology have improved outcomes for patients with cardiovascular diseases, less is known about circadian rhythms in breathing.

Chronic heart failure (CHF) is a condition characterized by disordered breathing, intermittent hypoxia, exercise intolerance, and chronically elevated sympathetic tone (Morrissey et al. 2011). Patients with reduced ejection fraction CHF do not show circadian rhythms in blood pressure (Komori et al. 2016). Typically, in healthy humans, blood pressure decreases during the night by about 10–20% of the mean daytime values (Floras et al. 1978). CHF patients are more likely than the general population to show abnormal light/dark blood pressure rhythms, and the particular pattern of blood pressure rhythm appears to vary with the type of CHF (Komori et al. 2016). CHF with preserved ejection fraction shows a “riser” pattern in which blood pressure is greater during the sleeping period, and CHF patients with reduced ejection fraction fail to show light/dark variations in blood pressure (Komori et al. 2016). While circadian cardiovascular rhythms have been examined in CHF, no one has yet to assess circadian rhythms in breathing.

Respiratory function has been shown to vary with the light/dark cycle (Mortola 2004). While light/dark variations in breathing have consistently been observed using continuous monitoring of respiration (Mortola and Seifert 2002; Seifert and Mortola 2002), respiratory measurements taken at two discrete time points have produced conflicting results. Light/dark variations in resting breathing were detected in neonate rats (Saiki and Mortola 1995) but not in adult rats (Peever and Stephenson 1997) when observed at two time points. Peever and Stephenson (1997) assessed ventilation in Sprague-Dawley rats at two time-points and found that chemoreflex breathing to hypercapnia increased during the night, but resting breathing showed no change between the light and dark periods. Notably, the respiratory rates for these animals at rest were considerably fast (96 ± 6 bpm during the day and 103 ± 8 during the night), which may have masked differences between the light and dark periods. In our experience, respiratory rates of adult Sprague-Dawley rats are much faster after first placing the animal in the plethysmography chamber, and respiratory rates decline to around 50–70bpm during the day. It is unclear why the respiratory rates in the Peever and Stephenson study remained elevated. In the current study, we assessed resting and chemoreflex breathing using whole body plethysmography at two time points, separated by about 12hrs, in a sham operated control group and a group with CHF induced by coronary artery ligation. Subsequently, light/dark variations in spontaneous exercise were also assessed using a running wheel.

1.2 Methods

1.2.1 Animals

Adult male Sprague Dawley rats (300–450g) were used in this study. Animals were held under a 12:12 light/dark cycle with lights on at 6am. All procedures were approved by the Institutional Animal Care and Use Committee at the University of Nebraska Medical Center.

1.2.2 Whole body plethysmography

Tidal volume (V_T), respiratory frequency (fR), and minute ventilation (V_E) were all determined by whole body plethysmography. V_T was measured using a differential pressure transducer (Kent Scientific) and amplifier (MP45, Validyne Engineering Corporation) prior to recording through a Power Lab System (AD Instruments) and Lab Chart v8.1.5. Rats were given at least 45 min to acclimate to the chamber, and then exposed to a series of gas challenges (10% O_2 with 2% CO_2 , 5% CO_2 , 90% O_2 , 5% CO_2 with 90% O_2) with 3–5 min of recovery time in between in each challenge. Chemoreflex breathing was assessed during the day (8am–2pm) and during the night (8pm–2am). V_T was normalized to body weight (kg). Chemoreflex sensitivity was quantified by subtracting V_E during a given challenge (10% O_2 , 5% CO_2 , and 90% O_2 with 5% CO_2) from baseline V_E .

1.2.3 Coronary artery ligation

Heart failure was induced via coronary artery ligation (n=5) as previously described (Del Rio et al. 2013). Sham animals underwent thoracotomy without coronary artery ligation (n=5). Six weeks following surgery, heart failure was verified using echocardiography (Vevo 770; Visualsonics). Two-dimensional short-axis view of the left ventricle was used to assess end-diastolic and end-systolic diameter as well as end-diastolic and end-systolic volume of the left ventricle. Animals with an ejection fraction <45% were considered to be in heart failure.

1.2.4 Scurry wheel

Voluntary exercise was assessed using Scurry Wheel (Lafayette Instruments, Model 80859S) which consists of a running wheel (inner diameter 35.56cm; width 10.92cm) inside a cage (40.64 × 50.80 × 20.96 cm). The scurry wheel was connected to an 8 channel Powerlab (AD Instruments), and wheel rotations were recorded in LabChart v8.1. To reduce the effects of the novel environment on behavior, each animal was given 24 hrs to acclimate to the cage. Animals had ad libitum access to food and water during the scurry wheel experiment. One heart failure animal died before completing the scurry wheel, reducing the n for the CHF group to 4.

1.2.5 Blood pressure

To assess light/dark variations in blood pressure, a group of animals were implanted with blood pressure telemetry (Datasciences International, either PA-C40 or HD-S10) in the left femoral artery. Surgical implantation occurred under 2–5% isoflurane balanced with oxygen, and animals were given at least 2 wks to recover. Following recovery from telemetry implantation, these animals underwent either coronary artery ligation (n=3) or a sham surgery (n=4), as described above. At least two weeks after the thoracotomy, these animals were placed in the cage with the running wheel, as described above, and blood pressure was recorded along with running wheel rotations. Segments of the blood pressure recordings in which the animal remained at rest for an extended period of time (>25 min) were selected to assess light/dark variations in blood pressure.

1.2.6 Statistics

All data are presented as mean \pm SEM, unless otherwise noted. One-way or two-way ANOVAs with Fisher's LSD used for pairwise comparisons were used to analyze the data. $p < 0.05$ was considered statistically significant.

1.3 Results

1.3.1 Echocardiographic data

Bodyweights between the sham (366 ± 12) and CHF (339 ± 7) groups did not differ ($p=0.08$). Differences were seen in cardiac structure and function (Table 1). The CHF group had greater end-diastolic ($p < 0.01$) and end-systolic ($p < 0.01$) volumes, greater end-diastolic ($p=0.02$) and end-systolic ($p < 0.01$) diameters, and lower ejection fraction ($p < 0.0001$) and fractional shortening ($p < 0.0001$). Two animals that were infarcted had ejection fractions greater than 45%, so they were excluded from the study.

1.3.2 Circadian rhythms in resting and chemoreflex breathing

Resting and chemoreflex breathing was assessed using whole-body plethysmography at two discreet time points, separated by approximately 12 hrs. The sham-operated control group displayed light/dark variations in V_E when breathing room air under baseline ($p=0.040$) and post-baseline ($p=0.038$) conditions (Fig 1A). The sham group also showed light/dark differences in V_E during chemoreflex breathing, specifically hypoxia ($p < 0.001$), hypercapnia ($p < 0.001$), and hyperoxia with hypercapnia ($p < 0.001$)(Fig 1A). In contrast, chronic heart failure eliminated circadian rhythms in resting breathing under the baseline ($p=0.57$) and post-baseline ($p=0.32$) conditions as well as during chemoreflex breathing (hypoxia $p=0.87$, hypercapnia $p=0.07$, and hyperoxia with hypercapnia $p=0.47$)(Fig 1A). There was also a main effect of heart failure status ($p < 0.001$) with the CHF group displaying greater V_E than the sham control group (Fig 1A).

Analysis of fR data showed a main effect of heart failure status ($p < 0.001$) with the CHF showing greater respiratory rates and a main effect of time ($p=0.02$) with respiratory rates increasing during the dark (Fig 1B). The sham group showed light/dark differences in fR under resting conditions (baseline: $p=0.02$; post-baseline: $p=0.04$) and in response to hypoxia ($p=0.01$), but not in response to hypercapnia ($p=0.54$), hyperoxia ($p=0.22$), or hyperoxia with hypercapnia ($p=0.15$)(Fig 1B). Respiratory rate did not differ between light and dark periods in the CHF group ($p=0.16$)(Fig 1B).

CHF animals had greater V_T than did sham animals ($p < 0.05$), but no main effect of time ($p=0.06$) or interaction ($p=0.48$) was seen (Fig 1C).

1.3.3 Circadian rhythms in chemoreflex sensitivity

Chemoreflex sensitivity was also assessed in sham and CHF animals during the light and dark periods. The data showed a statistically significant time x condition interaction ($p=0.005$), with the sham group displaying greater sensitivity during the dark compared to during the light period ($p=0.01$) while the CHF group showed no difference in chemoreflex sensitivity between the day and the night ($p=0.12$) (Fig 2).

1.3.4 Circadian rhythms in voluntary exercise

Voluntary locomotor activity was assessed using a running wheel, following a 24hr period in which each animal acclimated to the novel cage containing the running wheel (Fig 3). The total number of rotations were determined for the light and for the dark periods. Wheel running showed a main effect of time ($p < 0.001$) with no main effect of heart failure status ($p = 0.69$) or interaction ($p = 0.73$). The sham group ran 128 ± 14 rotations during the light period and 582 ± 241 rotations during the dark period ($p < 0.05$). The CHF group also ran more during the dark period (425 ± 60 rotations) compared to during the light period (124 ± 42 rotations) ($p < 0.05$). However, there was no difference the sham and CHF groups.

1.3.4 Circadian rhythms in blood pressure

Circadian rhythms in blood pressure and voluntary exercise were assessed in rats with chronically implanted telemetry. Mean arterial pressure (MAP) differed between the light and dark periods in the sham (light, 102 ± 2.13 mmHg; dark, 111 ± 3.49 mmHg; $p = 0.03$) but not in the CHF group (light, 97 ± 3.80 mmHg; dark, 85 ± 1.36 mmHg; $p > 0.05$). Similarly, systolic pressure was greater during the dark compared to the light in the sham group (light, 128 ± 1.14 mmHg; dark, 136 ± 1.62 mmHg; $p = 0.03$) but not the CHF group (light, 119 ± 8.96 mmHg; dark, 123 ± 9.42 mmHg; $p > 0.05$). Diastolic pressure did not differ between the light and dark periods in either the sham (light, 95 ± 4.86 mmHg; dark, 93 ± 4.6 mmHg; $p = 0.75$) or CHF group (light, 85 ± 1.36 mmHg; dark, 88 ± 2.93 mmHg; $p = 0.82$). The sham and CHF groups did not differ from each other in MAP ($p = 0.14$), systolic pressure ($p = 0.21$), or diastolic pressure ($p = 0.22$).

1.4 Discussion

Respiratory function has been shown to have a circadian periodicity and vary with the light/dark cycle (Mortola 2004). A previous study that acquired respiratory measurements at discrete time points did not find light/dark differences in resting breathing in adult Sprague-Dawley rats (Peever and Stephenson 1997); however, we assessed breathing during the light and dark periods, and we detected light/dark variations in V_E in both resting and chemoreflex breathing as well as light/dark differences in chemoreflex sensitivity in the control group. The elevated respiratory rates (~ 90 bpm) of the former study may have masked light/dark variations in resting breathing. In contrast, the control group of the current study showed respiratory rates of 70 ± 3.87 bpm during the light period and 81 ± 3.73 bpm during the dark. The current study demonstrates that measurements of respiratory function taken at discrete time points can be used to detect light/dark variations in breathing in healthy adult rats. These findings are consistent with previous studies that found greater HVR (Seifert and Mortola 2002) and HCVR (Peever and Stephenson 1997) during the dark in rats.

In addition to finding circadian rhythms in resting and chemoreflex breathing in healthy adult rats, we also assessed these rhythms in CHF rats with reduced ejection. To our knowledge, this is the first study to assess circadian rhythms in respiration in CHF. Light/dark variations in respiration and arterial pressure appear to be abolished in CHF with reduced ejection fraction. Our study corroborates previous work on blood pressure rhythms

in human heart failure patients (Komori et al. 2016) and mice with myocardial infarctions (Mousa et al. 2014). The current study expands previous findings in showing that CHF also eliminates respiratory circadian rhythms. Although CHF rats did not show a circadian rhythm in breathing, chemoreflex sensitivity, or blood pressure, the CHF group showed light/dark variation in voluntary exercise. These results suggest that CHF impairs circadian rhythmicity of autonomic function with respect to neural control of breathing and cardiovascular function before significantly impacting circadian variations in behavioral locomotor activity.

Also, surprisingly, the CHF group exercised the same amount as the sham control group. The reason for this finding is unclear. Exercise intolerance in CHF appears to be mediated largely by skeletal muscle myopathy (Piepoli and Crisafulli 2014). Thus, it is conceivable that while ejection fraction was decreased and respiratory function was abnormal, skeletal muscle myopathy was not sufficiently advanced to impair locomotor activity.

The mechanism underlying the respiratory circadian rhythm in the control group and the lack of light/dark variations in the CHF group have not been determined but 24hr variations in carotid body chemoreceptor sensitivity is a promising target. A series of ex vivo experiments in the rat carotid body suggest that carotid body sensitivity varies between the light and dark periods (Chen et al. 2005; Tjong et al. 2006). The firing rate of the rat carotid body increases significantly in response to hypoxia increases in the presence of melatonin (Chen et al. 2005; Tjong et al. 2006), a hormone that is released during the night in both humans (Grivas and Savvidou 2007) and rats (Lynch et al. 1984). Altered carotid body function may explain the lack of light/dark variations in breathing in CHF. While the responsiveness of the carotid body in the presence of melatonin may promote light/dark variations in healthy controls, carotid body function is exaggerated at rest and in response to hypoxia and hypercapnia in CHF (Andrade et al. 2015). The already heightened activity of these cells during the light and dark periods may mask light/dark differences in resting and chemoreflex breathing. Future studies should assess the role of melatonin in modulating resting and chemoreflex breathing during the light and dark periods in healthy controls and explore how this process may be altered in CHF.

Acknowledgements

The authors would like to acknowledge Mary Ann Zink and Kaye Talbitzer for their technical assistance in completing this project.

This work was supported by NIH PO1-HL062222-17.

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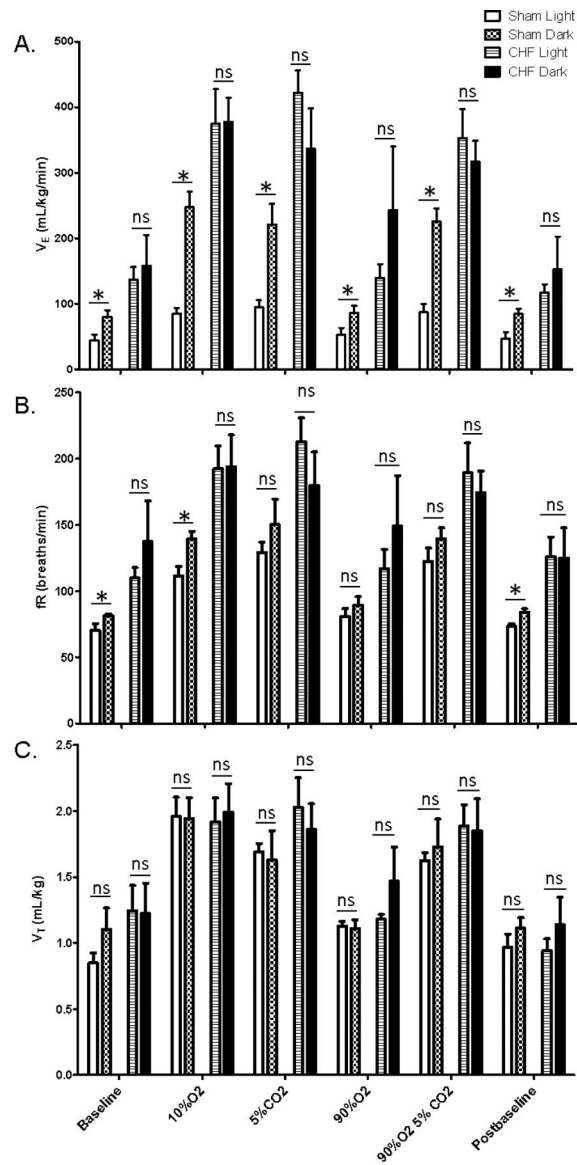


Fig 1. Light/Dark variations in resting and chemoreflex breathing

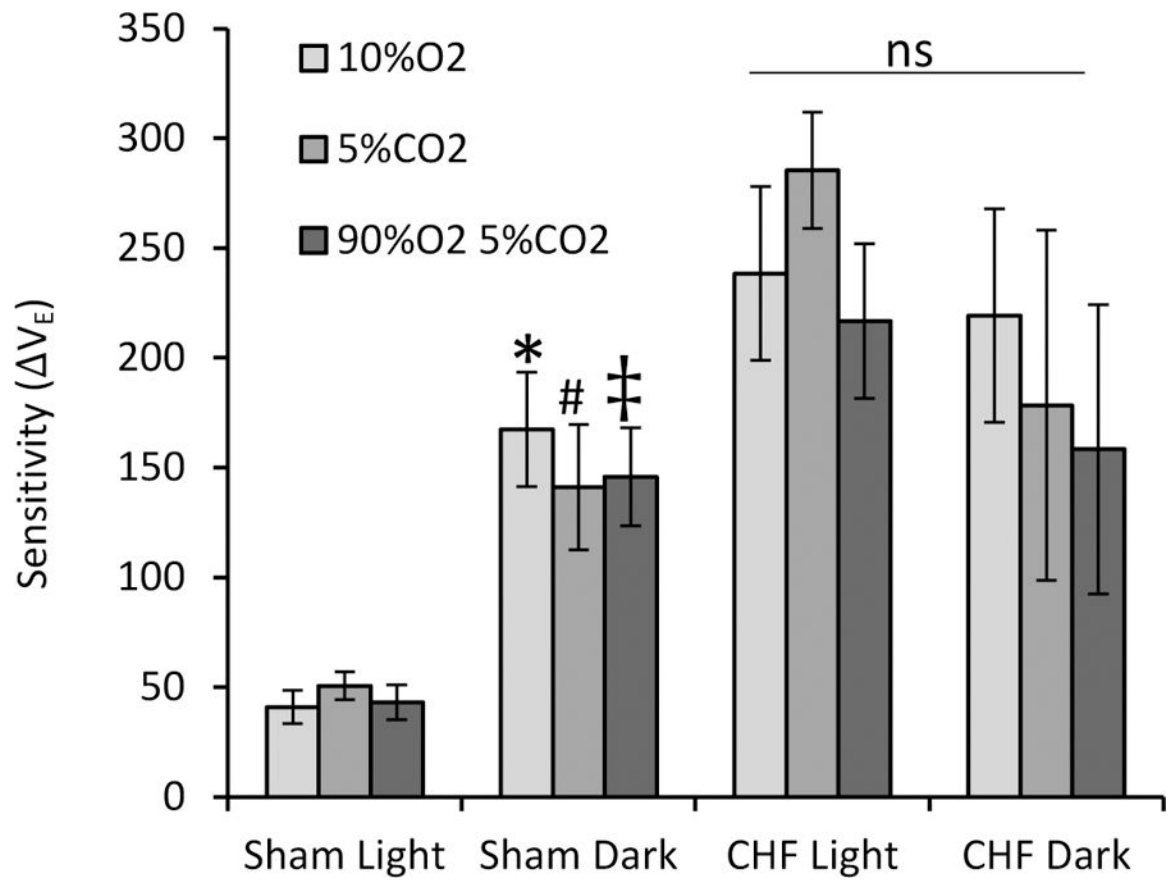


Fig 2. Chemoreflex sensitivity

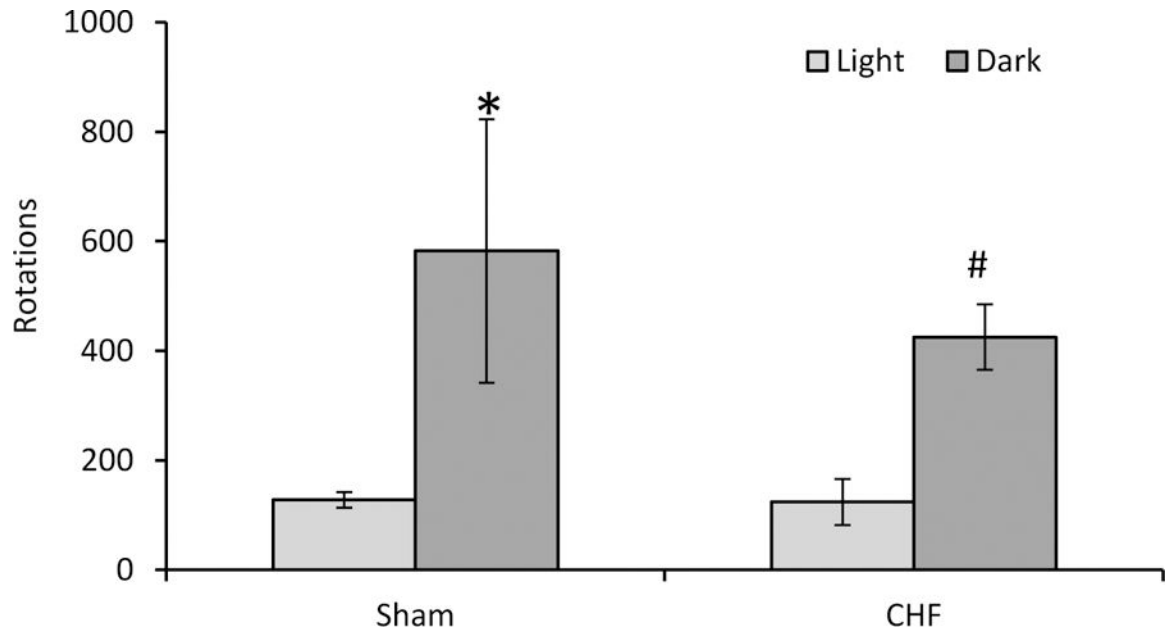


Fig 3. Voluntary exercise

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Table 1

Cardiac Function

Condition	BW (g)	LVEDD (mm)	LVESD (mm)	LVEDV (μ L)	LVESV (μ L)	SV (μ L)	EF (%)	FS (%)
Sham	441 \pm 27.8	0.83 \pm 0.06	0.56 \pm 0.05	132 \pm 25	44 \pm 8	88 \pm 17	66.8 \pm 3.55	31.8 \pm 3.24
CHF	484 \pm 16.9	1.21 \pm 0.07 *	1.03 \pm 0.07 *	353 \pm 51 *	228 \pm 39 *	123 \pm 12 *	36.4 \pm 2.65 *	15.5 \pm 1.26 *

* statistically different from sham; sham, n=5; chf n=5

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