

The circadian pacemaker generates similar circadian rhythms in the fractal structure of heart rate in humans and rats

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Aims Adverse cardiovascular events in humans occur with a day/night pattern, presumably related to a daily pattern of behaviours or endogenous circadian rhythms in cardiovascular variables. Healthy humans possess a scale-invariant/fractal structure in heartbeat fluctuations that exhibits an endogenous circadian rhythm and changes towards the structure observed in cardiovascular disease at the circadian phase corresponding to the time of the broad peak of adverse cardiovascular events (at about 10 AM). To explore the relationship between the rest/activity cycle, endogenous circadian rhythmicity, and cardiac vulnerability, we tested whether the fractal structure of heart rate exhibits a similar circadian rhythm in a mammalian species that is nocturnally active (Wistar rats) compared with diurnally active humans, and how this fractal structure changes after lesioning the circadian pacemaker (suprachiasmatic nucleus, SCN) in rats.

Methods and results Analysis of heart rate data collected over 10 days in eight intact and six SCN-lesioned Wistar rats during constant darkness revealed that: (i) as with humans, rats exhibit an endogenous circadian rhythm in the scaling exponent characterizing the hourly fractal structure of heart rate ($P = 0.0005$) with larger exponents during the biological day (inactive phase for rats; active phase for humans); (ii) SCN lesioning abolished the rhythm in the fractal structure of heart rate and systematically increased the scaling exponent ($P = 0.01$).

Conclusion Rats possess a circadian rhythm of fractal structure of heart rate with a similar temporal pattern as previously observed in humans despite opposite rest/activity cycles between the two species. The SCN imparts this endogenous rhythm. Moreover, lesioning the SCN in rats results in a larger scaling exponent, as occurs with cardiovascular disease in humans.

1. Introduction

Healthy heartbeat fluctuations possess a scale-invariant/fractal structure characterized by long-range power-law correlations over a range of time scales.^{1–3} These scale-invariant correlations persist during different behaviours and in varied environments, but change with autonomic blockade^{4–6} and under pathological conditions such as congestive heart failure.^{7–9} In addition, these scale-invariant correlations provide one of the most sensitive heart rate variability (HRV) biomarkers for predicting survival rates in patients following stroke¹⁰ and acute myocardial

infarction.¹¹ These findings suggest that the scale-invariant correlations are intrinsic patterns of cardiac dynamics with relevance to cardiovascular health and survival.

There is considerable epidemiological evidence of a day/night pattern in the timing of stroke, myocardial infarction, ventricular arrhythmias, and sudden cardiac death with a broad peak at about 10 AM.¹² However, it is not known whether this pattern is caused by the day/night patterns of behaviours or endogenous circadian rhythms in relevant physiological variables.¹² In mammals, endogenous circadian rhythms are generated by the circadian pacemaker located in the suprachiasmatic nucleus (SCN) of the hypothalamus.^{13,14} We recently discovered that the scale-invariant/fractal structure in human heartbeat fluctuations exhibits an endogenous circadian rhythm, independent of scheduled behaviours such as the sleep/wake cycles¹⁵ and independent

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from overall activity level while awake.¹⁶ In healthy subjects, the statistical index that quantifies the fractal structure was highest at the specific circadian phase that corresponds to the time of day when adverse cardiovascular events are most frequent,^{15,16} moving this index closer to that observed in cardiovascular disease.⁷⁻⁹ These findings lead us to hypothesize that the SCN, which generates circadian rhythms in many physiological functions including the cardiovascular system,¹⁷⁻²¹ may play a role in the timing of adverse cardiovascular events.^{15,16} In this study, we explored endogenous circadian rhythmicity in the fractal structure of heart rate and its relationship with the rest/activity cycle by testing: (i) whether or not there exists a circadian rhythm in the fractal structure of heart rate in a mammalian species that is nocturnally active; (ii) whether the phase relationship between the rest/activity cycle and the circadian rhythm of fractal structure of heart rate is altered in nocturnally active mammals relative to that in humans; and (iii) how the degree and circadian rhythmicity of the fractal structure of heart rate change after lesioning the circadian pacemaker.

2. Methods

2.1 Protocol

Recordings were made in nocturnally active Wistar rats during constant dark conditions (DD protocol) as described previously.²² Each rat lived individually in a cage with a dimension of $39 \times 38 \times 38$ cm³. Two groups of rats were used: eight control rats, and six rats following successful SCN lesion. Preceding the DD protocol, all rats underwent 10 light/dark cycles (12 h light, 100 lux; 12 h dark, 0.1 lux; LD protocol). The LD protocol ensured that the circadian pacemaker and rest/activity cycles were synchronized to the light-dark cycles (i.e. 12 h inactive phase and 12 h active phase) prior to the DD cycle. The investigation was approved by the Animal Care Committee of the Royal Netherlands Academy of Arts and Sciences and conformed to the *Guide for the Care and Use of Laboratory Animals* published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996).

2.2 Suprachiasmatic nucleus lesion

Standardized surgical techniques were used to ablate the SCN as described previously.²² Briefly, 30 rats were anaesthetized (Hypnorm; 0.8 mL/kg i.m.), mounted in a stereotactic instrument, and electrode tips (0.2 mm diameter) were placed bilaterally in the SCN and heated to 80°C for 1 min. To avoid inclusion of rats with incomplete lesions, the day/night rhythms in drinking water were investigated at least 1 month after surgery, and vasoactive intestinal polypeptide (VIP) and vasopressin (VP) within the SCN in perfusion-fixed brains were quantified.^{23,24} Rats with rhythms in drinking behaviour, or with VIP or VP present, were excluded from analysis. Seven of the 30 rats met both behavioural and anatomical verification of complete SCN lesion. These SCN-lesioned (SCNx) rats were recorded throughout the same LD and DD protocols as the control rats. During DD, there were technical difficulties in the data collection of one SCNx rat, so data from only six SCNx rats are presented.

2.3 Data acquisition

This paper presents a re-analysis of previously published data.²² For the collection of heart rate data, a transmitter was implanted in each rat under anaesthesia (0.8 mL/kg i.m. Hypnorm and 0.4 mL/kg s.c. Dormicum) and secured to the inner muscle wall of the abdomen, one rostral and one caudal to the heart, as described

previously.²² After surgery, animals recovered for at least 2 weeks and then heart rate data were transmitted from the freely moving rat to a telemetry antenna underneath the cage. Every 4 min, heart rate was sampled at 500 Hz for 10 s and the average was stored. Core body temperature was also measured every 4 min. Long-range correlations (across 4–24 h) in locomotor data²⁵ and in heart rate data²⁶ from the same study have been published previously.

2.4 Data analysis

2.4.1 Scale-invariant/fractal structure in heart rate fluctuations

To quantify the scale-invariant/fractal structure in heart rate fluctuations, we used detrended fluctuation analysis (DFA) on hourly segments of heart rate data. DFA is designed to quantify correlations in signals that may be masked by underlying non-stationarities or trends.^{27,28} This method quantifies the detrended fluctuation function of heart rate at different time scales, n (see Supplementary material online for detailed procedures). A power-law functional form indicates self-similarity (scale-invariance). This power-law functional form is defined by a straight line on a log-log plot of $F(n)$ vs. time scale (bin width), yielding $F(n) \sim n^\alpha$. The parameter α , called the scaling exponent, quantifies the correlation properties in the signal: if $\alpha = 0.5$, there is no correlation in the fluctuations (random noise); if $\alpha < 0.5$, the signal is anticorrelated, where large heart rate values are more likely to be followed by small heart rate values; if $\alpha > 0.5$, there are positive correlations, where large heart rate values are more likely to be followed by large heart rate values (and vice versa for low heart rate values). For each rat and for each circadian bin (see 2.4.2 Circadian phase), we estimated the scaling exponent α over the same range of time scales, 4–40 min (Figure 2A). The variation of the scaling exponent α at different bins reveals the effect caused by circadian pacemaker.

To quantify the scale-invariant patterns in cardiac dynamics, most other studies have used continuous beat-to-beat intervals,^{4,27,29} but beat-to-beat data were unavailable in the current study. Instead, we analysed heart rate data of rats that were collected every 4 min on the basis of 10 s sampling (see 2.3 Data acquisition). Such difference in sampling frequency and variable (i.e. heart rate vs. inter-beat interval) can systematically affect the scaling exponent obtained by the DFA. To account for the sampling effect and enable the comparison of the current and previous studies, all scaling exponents presented in the current study were adjusted by adding 0.15 (see Supplementary material online for details).

2.4.2 Circadian phase

Core body temperature was used to determine the individual circadian periods of control rats during the DD protocol, with the start of the DD cycle (corresponding to the onset of the light period in the preceding LD protocol) being assigned circadian phase 0°. To appropriately align data from SCNx rats for comparison with control rats, the start of the DD cycle was again assigned circadian phase 0°, and the average circadian period of the control rats in the DD protocol, i.e. 24.1 h,²² was used for assignment of extrapolated circadian phase bins.

2.4.3 Statistical analysis

For data analysis, the circadian cycle (360°) was divided into nine non-overlapped circadian phase bins (equivalent to 40° or ~ 2.67 h per bin). To determine whether or not there exists an endogenous circadian variation in the heart rate-scaling exponent, data were normalized (deviation from the mean), and subjected to one-way ANOVA, separately for control and SCNx groups. To test for any interaction effects upon the scaling exponent between group and circadian phase (biological day vs. biological night), a two-way ANOVA was performed. Unpaired *t*-tests were used for the

comparisons of mean heart rate and scaling exponent between control and SCNx groups.

3. Results

3.1 Endogenous circadian rhythm of cardiac dynamics in rats

During DD, there was a significant circadian rhythm in heart rate in control rats (Figure 1; black squares). Mean heart rate was 341 b.p.m. (SEM = 4 b.p.m.), and the average peak-to-trough circadian fluctuation was ~40 b.p.m. Heart rate was lower during the biological day (inactive phase in these animals, corresponding to the light periods in the preceding LD protocol). Similarly, during DD, there was a significant circadian rhythm in the scaling exponent of heart rate fluctuations in control rats (Figure 2B; black squares). The average adjusted scaling exponent α for control rats across all circadian phases was 1.03 ± 0.04 (SEM). Notably, there was a substantially larger α exponent at circadian phases 0° – 180° , corresponding to the inactive biological day in these nocturnal animals when compared with the biological night ($\Delta\alpha \approx 0.15$; $P = 0.0005$). The α exponent is also larger during the biological day in diurnally active humans (Figure 2C).^{15,16} Taken together, these results suggest that the circadian rhythm in α is independent of activity level because the relationships between α and activity are reversed between these species.

3.2 Suprachiasmatic nucleus lesion abolished circadian rhythms in heart rate and cardiac dynamics

The circadian rhythms of core temperature, activity, and heart rate were abolished by SCN lesion,²² with the heart rate data shown in Figure 1 (circles). The group average heart rate was not significantly different between the two groups (SCNx 346 ± 7 b.p.m., mean \pm SEM; control rats 341 ± 4 b.p.m.; $P = 0.47$). Similarly, the circadian rhythm in the scaling exponent α was abolished by SCN lesion (Figure 2B, circles), indicating that the SCN generates a circadian rhythm in scale-invariant cardiac dynamics. Overall,

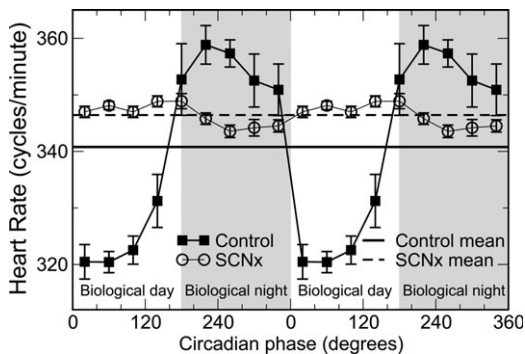


Figure 1 Circadian rhythms in the group average of mean heart rate during constant dark conditions. In control rats, heart rate exhibits a significant circadian variation (black square: $P < 0.0001$) with lower values at 0° – 180° [corresponding to biological day (inactive phase)] and higher values at 180° – 360° [grey region; corresponding to biological night (active phase)]. No circadian rhythms occur in the heart rate of suprachiasmatic nucleus-lesioned rats (open circle: $P > 0.45$). The mean heart rate across all circadian phases is slightly higher in suprachiasmatic nucleus-lesioned rats (dashed line) than in controls (black line), but the difference is not significant ($P > 0.47$). Data are double-plotted to better visualize circadian rhythmicity.

the scaling exponent α was larger in SCNx rats than in control rats (SCNx $\alpha = 1.20 \pm 0.04$, mean \pm SEM; control 1.03 ± 0.04 ; $P = 0.01$). The group difference in α was most pronounced during the biological night ($P = 0.003$, Figure 2B). ANOVA confirmed the phase dependence of the α difference between the control and SCNx rats ($P = 0.01$).

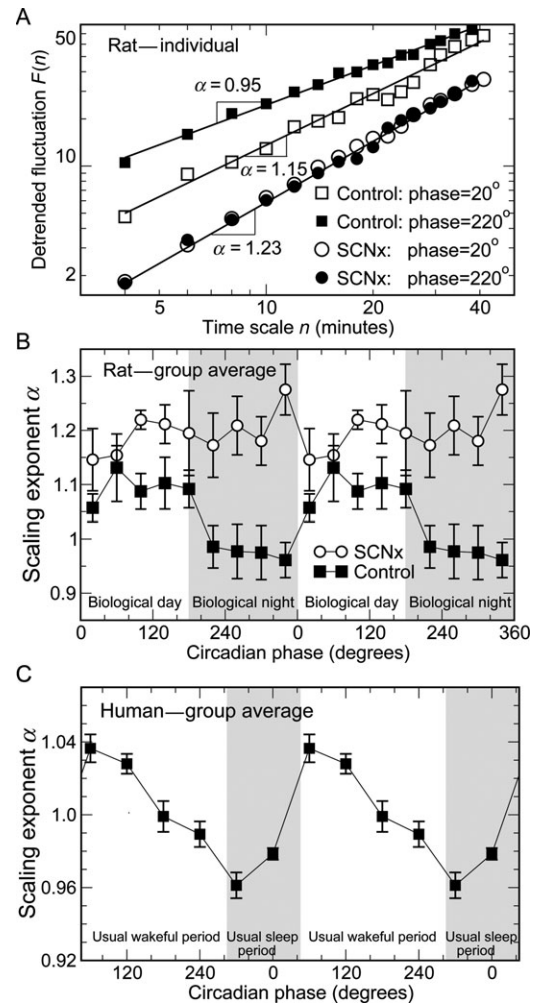


Figure 2 Circadian rhythms in the correlation property of heart rate dynamics. (A) Detrended fluctuation analysis results for a control rat at two different circadian phases [biological day (open squares) and biological night (filled squares)] and for an suprachiasmatic nucleus-lesioned rat at the same extrapolated circadian phases (open and closed circles). (B) Significant circadian rhythms are observed in the group average of the scaling exponent α in control rats (black squares). α is larger during the biological day (inactive phases 0° – 180°) than during the biological night (active phases 180° – 360°). There was no significant circadian rhythm in α of suprachiasmatic nucleus-lesioned rats (circles), yet α is significantly larger in suprachiasmatic nucleus-lesioned rats than in controls across all circadian phases. The grey region indicates circadian phases corresponding to the biological night for rats. (C) Similar circadian rhythms in the group average of the scaling exponent α were also observed in healthy humans (adapted from Hu et al.¹⁵). As in control rats, core body temperature of each human subject was used to determine the individual circadian period. In human data, circadian phase 0° was assigned to the time of the fitted minimum core body temperature (corresponding to about 5 AM),¹⁵ whereas phase 0° was assigned to the start of the DD cycle in control rats (corresponding to the onset of the light period in the preceding LD protocol; see Methods). The grey region indicates circadian phases corresponding to the usual sleep period of human subjects. The results of α in (B) and (C) are double-plotted to better visualize circadian rhythmicity.

4. Discussion

This study yielded four main findings. (i) As with humans, there exists a circadian rhythm in the fractal structure of heart rate fluctuations in rats. This suggests that there may be a common scale-invariant heart rate control mechanism in mammals. (ii) As with humans, the scaling exponent characterizing the fractal structure of heart rate in rats is larger during the biological day compared with the biological night, i.e. the period when it is normally dark (although the rats and humans were studied in constant darkness or dim light conditions). Since the average neuronal activity of the central circadian pacemaker is normally in synchrony with the environmental day/night (or light/dark) cycle in both nocturnal and diurnal species, peaking during the biological day (or light phase when under L/D conditions),^{21,30–34} these findings suggest that the phase relationship between the endogenous neuronal activity cycle of the SCN and the circadian cycle of the fractal structure of heart rate is similar between the two species. In contrast, the phase relationship between the rest/activity cycle and the circadian cycle of the fractal structure of heart rate is clearly different between these species. This suggests that the circadian variation in the fractal structure in heart rate fluctuations is not mediated via changes in the mean level of activity or heart rate. (iii) Lesioning the SCN in rats completely abolished the circadian rhythm in the fractal structure of heart rate. This demonstrates that in mammals, the SCN is critical for the circadian rhythm in scale-invariant heart rate control. (iv) The scaling exponent α is systematically larger when the SCN is ablated. This suggests that in control rats, the SCN normally decreases α throughout all circadian phases with a stronger influence during the biological night when compared with during the biological day (as discussed in Section 4.3).

4.1 Potential mechanisms underlying changes in scale invariance of heart rate fluctuations across the circadian cycle

The scale-invariant/fractal heart rate pattern reveals a complex temporal organization in heart rate fluctuations and provides complementary dynamical information of heart rate control that is independent of traditional HRV parameters used for the assessment of mean heart rate and sympatho-vagal activity.^{10,35–39} This is exemplified by the observations that vagal tone decreases in humans and increases in rats at circadian phases corresponding to the biological day, and vice versa during the biological night,^{20,21} but the scaling exponent characterizing heart rate scale invariance has similar circadian rhythm profiles in humans and rats, with larger exponents during the biological day (Figure 3). Mathematical models of physical systems reveal that such scale-invariant patterns can be explained by interactions between multiple control components that affect the overall system at different time scales.^{40–42} Thus, scale invariance in heartbeat fluctuations could emanate from a variety of interacting influences on heart rate including sympathetic and parasympathetic nervous system activity, circulating catecholamines and cortisol, changes in temperature, and intrinsic properties of the heart itself.⁴³ The SCN generates and coordinates circadian rhythms in many physiological and behavioural

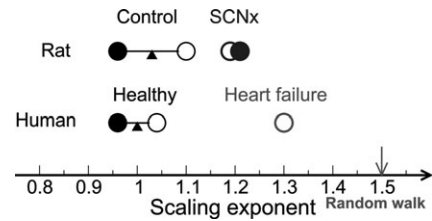


Figure 3 Illustration of influences of the suprachiasmatic nucleus on scale-invariant correlations of heart rate dynamics. The scale-invariant correlations in healthy humans and control rats, as characterized by a scaling exponent α , are similar and exhibit endogenous circadian rhythms, with larger α at circadian phases corresponding to the biological day (open circles) and smaller α at circadian phases corresponding to the biological night (solid circles). Following suprachiasmatic nucleus lesion, the circadian rhythm in the scale-invariant correlations in rats was abolished, as indicated by the similar α values at all circadian phases. In addition, suprachiasmatic nucleus lesion causes a significant increase in α value, indicating that the correlations change towards the direction of a random walk—an integration of white noise that is associated with a simple process without any underlying feedback control and characterized by $\alpha = 1.5$ (arrow). The scale-invariant cardiac correlations in cardiac diseases such as congestive heart failure are characterized by higher values of the scaling exponent $\alpha \approx 1.3$ during daytime.⁷

functions.^{44,45} Thus, the SCN could exert its influences on scale-invariant heart rate control via direct projections influencing autonomic and humoral activation of the heart,^{19,21,46–48} or via indirect pathways whereby circadian-related changes in activity or temperature could affect heart rate fluctuations.

In our previous human studies, the timing of the circadian peak in the scaling exponent characterizing the fractal structure of heart rate is not in alignment with the circadian peak in mean heart rate and motor activity levels,¹⁵ and the circadian rhythm of α persists when circadian rhythms of motor activity were experimentally abolished using a constant routine protocol.¹⁶ These findings clearly indicate that the circadian rhythm of the HRV fractal structure in humans is independent of circadian influence on activity.

4.2 Limitations

In humans, heart beat fractal patterns at very small time scales (<10 beats) and at larger time scales (from 11 to ~10 000 heartbeats) differ and provide complementary information about cardiac control.^{7,49} However, studies of heart rate in rats have not reported any significant difference in the fractal patterns at different time scale ranges.^{29,50} The scaling exponent characterizing the patterns in rats is identical to that in humans at time scales >11 beats. In this study, we note that since only average heart rates were stored every 4 min, we cannot study fractal structure in heart rate at time scales close to a few heartbeats.

In this study, we focused on the modulation on cardiac scale invariance at different circadian phases. In order to examine changes across 24 h, the bin width must be sufficiently small to ensure capturing the peaks and troughs. This approach does not allow us to study scale invariance at large time scales (i.e. changes at different circadian phases can be smoothed out when considering a larger time scale). Evidence that the SCN is involved in cardiac scale-invariant control across a larger time scale (from ~4 to 24 h) has been previously published.²⁶

Currently, we could not identify the underlying pathways responsible for the SCN influence on the scale-invariant heart rate control. The SCN influences the autonomic nervous system,^{18,20,46,51,52} and sympathetic and parasympathetic blockade or autonomic dysfunction can alter scale-invariant cardiac dynamics.^{4–6} In the current study, we could not assess the involvement of the branches of autonomic nervous system to the circadian modulation of the fractal structure of heart rate because beat-to-beat recordings were not available for the estimation of standard HRV indices.

4.3 Significance of changes in scale invariance of heart rate fluctuations across the circadian cycle

Scale-invariant regulation of many physiological variables appears to provide greater adaptability and biological advantage compared with simple homeostatic control.⁵³ The scale-invariant structure of heart rate fluctuations changes under pathological conditions^{7–9} and provides one of the most sensitive HRV biomarkers for predicting survival rates in patients after stroke¹⁰ and acute myocardial infarction.¹¹ Thus scale-invariant correlations are intrinsic patterns of heart rate dynamics, with relevance to cardiovascular health and survival.

Almost identical scale-invariant HRV patterns are observed in human, dog, mouse, rat, swine, and sheep,^{1–3,29,54–57} suggesting that there may exist a common scale-invariant cardiac control mechanism in mammals. In humans, epidemiological studies reveal a 24 h daily pattern in adverse cardiac events with a broad peak at ~9–11 AM,^{58–60} presumably caused by the day/night patterns of behaviours or internal influences from the circadian pacemaker.¹² We previously found in healthy humans an increase in the scaling exponent α at the circadian phases corresponding to the time window of highest cardiac risk (9–11 AM).¹⁵ In humans, cardiovascular diseases are associated with a larger α value compared with healthy controls, thus we speculated that the time of peak cardiac risk may be partly caused by an endogenous circadian-mediated increase in α .¹⁵ In the present study, we found that nocturnally active rats possess an endogenous circadian rhythm in scale-invariant cardiac control, and the phase relationship between circadian changes in α and the normal day/night cycle is similar between humans and rats.^{21,30–34} Using an animal model of cardiovascular disease, it would be worthwhile to determine whether such animals exhibit an increased exponent α as in diseased humans, and whether or not these animals exhibit an endogenous circadian rhythm in adverse cardiovascular events. Furthermore, although we speculate that the endogenous circadian influence on the scale-invariant cardiac control may contribute to an underlying day/night pattern of cardiac risk, it is likely that behavioural factors such as motor activity levels also provide a major contribution to cardiac vulnerability.¹² Future studies could test the relative contribution of dynamics vs. mean levels of heart rate and other physiological markers to cardiac risk in nocturnal mammals.

SCN lesion systematically increased the scaling exponent α , suggesting an altered scale-invariant heart rate control in the SCNx rats that resembles humans under pathological conditions (Figure 3). This finding raises the intriguing hypothesis that the SCN, as a master clock promoting

adaptation of body functions to daily rest and activity, may have a protective role in heart rate control. This is consistent with the observation that a decreased activity of the SCN is associated with a history of hypertension, which is a major risk for cardiovascular diseases.⁶¹ Such a hypothesized protective role of the SCN in heart rate control could also provide an explanation for previous observations that disturbing the circadian system is associated with increased risk of cardiovascular diseases^{62–64} and increased morbidity and mortality.^{65,66} Further studies will be required in order to test this hypothesis.

Supplementary material

Supplementary material is available at *Cardiovascular Research* online.

Conflict of interest: none declared.

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