Heart Rate Variability Analysis Reveals a Non-monotonic Relationship between Humanin Concentration and Cardiac Autonomic Regulation

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Abstract

Oxidative stress (OS) has been shown to have a negative effect on the autonomic nervous system (ANS) and on ANS modulation of heart rate. Mitochondrial ATP production is the main source of reactive oxygen species (ROS) and hence the regulation of ROS becomes an important issue in maintaining optimal ANS functionality. Humanin (HN), a mitochondrial-derived peptide, plays an important role in lowering OS. Sympathovagal balance was assessed in 124 healthy participants through heart rate variability (HRV) analysis and compared across changes in HN concentrations divided into quintiles, with values of HN ranging from 64.6 to 343.2 pg/mL.

Significant differences included various frequency domain and nonlinear HRV parameters, particularly between first and fourth HN quintiles with p values < 0.001 for recurrence plot analysis (RPA), detrended fluctuation analysis (DFA) α1 and Poincaré plot ratio SD1/SD2. The results revealed non-monotonic relationships between measures of HRV and HN concentration. A mitohormetic type of relationship was observed with HRV features increasing and then decreasing with increasing HN concentration. These results are consistent with previous findings of the importance of HN levels in regulating OS and extend these by revealing a concomitant effect on the modulation of cardiac rhythm by the ANS.

1. Introduction

Cardiac autonomic neuropathy (CAN) is characterized by damage to the autonomic nervous system (ANS), resulting in abnormal visceral function including heart rate control[1]. Sudden cardiac death may be a consequence of abnormal autonomic modulation of cardiac rhythm and associated with oxidative stress (OS) [2–4]. The mitochondrial electron transport chain generates over 90% of intracellular reactive oxygen species (ROS), thereby representing the primary source of OS [2]. While ROS are essential for a number of physiological processes, they can induce damage to the ANS if left unchecked. The resulting parasympathetic (PNS) suppression and sympathetic (SNS) stimulation is associated with changes in heart rate variability (HRV).

To effectively regulate excess intracellular ROS produced by the mitochondria, antioxidant defense systems are required [2].

Originally discovered as a neuroprotective peptide in Alzheimer's disease, humanin (HN) is a mitochondrial derived peptide (MDP) that imparts cytoprotective effects by inhibiting apoptosis and lowering OS induced by the mitochondria [5]. HN activity may therefore also impact ANS modulation of cardiac rhythm.

HRV analysis includes time-domain, frequency-domain and nonlinear measurements [6]. As such, HRV has been utilized to investigate the relationship between OS and autonomic regulation in health and disease including cardiovascular disease [7,8,4] . The aim of the present study was to examine the relationship between HN and autonomic modulation through short-term HRV analysis in order to provide a context for use of antioxidant therapy including humanin analogs that decrease risk of sudden cardiac death due to oxidative stress.

2. Methods

Data was obtained from a rural diabetes screening clinic at Charles Sturt University (CSU) (DiabHealth), Albury, Australia, between the years 2002 to 2015. The study was approved by the CSU Human Research Ethics Committee, and written informed consent was obtained from all participants.

Blood pressure measurements (lying systolic (SBP) and diastolic blood pressure (DBP)), body mass index (BMI) and lipid profiles including high density lipoprotein (HDL), low density lipoprotein (LDL), triglycerides and total cholesterol (TC) were obtained [9]. Humanin levels were determined using a commercial ELISA kit detailed in [10]. Missing values (MVs) in the dataset were imputed using the mean or mode of data subsets, which are generated based on selector variables with the highest Information Gain criterion [11,12].

Following the application of inclusion and exclusion criteria, data from 151 participants were divided into quintiles based on HN values. The time elapsed between two successive R waves of the QRS complex (RR intervals) was obtained from 10 minute electrocardiogram recordings, and HRV features determined using Kubios HRV software [13]. Recordings with excessive outliers and ectopics were removed, leaving 124 participants for analysis. HRV features included the time domain measure root mean square of successive differences (rMSSD), frequency domain measures of high frequency power (HF) and the low frequency to high frequency ratio (LF/HF). Nonlinear measures included detrended fluctuation analysis (DFA) $\alpha1$ and $\alpha2$, Poincaré plot ratio SD1/SD2, sample entropy (SampEn) and recurrence plot analysis (RPA). RPA measures (determinism (DET), recurrence rate (REC) and divergence (DIV)).

The non-normal distribution of the data was log-transformed. Given that heart rate (HR) and respiration rate (RESP) have been shown to impact HRV reproducibility [14], differences in HR and RESP (Hz) were investigated between HN quintiles, and Pearson's r (ρ) was computed for HR and RESP with HRV parameters to assess correlations [15]. ANOVA or Welch's ANOVA was applied based on the results of Bartlett's test for equal variances. This was followed by Games-Howell *post hoc* test to detect significant differences while controlling family-wise error rate. All statistical analyses were performed using RStudio (1.4.1717) and significance set at $p<0.05$.

3.   Results

No significant differences were found between HN quintiles for HR, RESP, age, gender, BMI, blood pressure or lipid profile as revealed by ANOVA and χ^2 tests. The lipid profile was within the normal range for all groups, but BMI was elevated for all participants (BMI<20 kg/m^2). Number of participants within each of the HN quintiles and HN concentration distribution can be seen in Table 1. Furthermore, weak correlations were found for HR and RESP with HRV metrics, as the largest coefficient

obtained for HR was with DIV at -0.46, and for RESP was -0.39 with HF,

Changes in the mean and standard errors of HRV parameters with HN quintiles are illustrated in Figure 1. Significant differences were found between HN quintile 1 with quintiles 3 and 4. Significant differences were found for DIV, SD1/SD2 and DFA $\alpha1$.

Similar patterns of change can be seen in the means of all HRV parameters. Up to quintile 3 or 4, an increase in RMSSD, HF, DIV, SD1/SD2 and SampEn can be observed. Values then decrease at either quintile 4 or 5. Similarly, LF/HF, REC, DET and DFA $\alpha1$ initially decreased before increasing for the 5th quintile.

In general, these graphs highlight the presence of non-monotonic relationships between HN and HRV measures in the form of U or inverted U-shaped curves.

Table 1. HN quintiles: Number of participants (N), minimum (Min) and maximum (Max) concentrations.

Quintile	N	Min (pg/mL)	Max (pg/mL)
1	28	64.6	148.4
2	21	150.6	192.9
3	24	194.8	230.6
4	24	230.6	242.6
5	27	251.0	343.2

4.   Discussion

The current study is the first to examine the relationship between cardiac autonomic modulation and levels of HN by analyzing HRV.

HN performs a variety of functions to maintain optimal mitochondrial function and levels of ROS [16]. HN has been shown to decrease with age [5], however, participants in the current study were matched in age, indicating that other factors lead to upregulation of HN such as the level of mitochondrial stress [16]. Decreased HN may reflect chronic stress leading to mitochondrial damage and loss of HN transcription. This, however, requires further investigation to fully elucidate the mechanisms at hand.

Given that no significant differences were found between quintiles in terms of HR and RESP, it is reasonable to assume that these rates do not have a particular influence on HRV metrics in the present study [17], therefore mathematical adjustment of HRV is unnecessary in this case.

Our results are in agreement with Fadaee et al. [7] who have investigated the relationship between HRV and OS , reporting a decrease in rMSSD and HF power with increasing OS burden, which in the current study would be interpreted as lower levels of HN as seen at quintiles 1 and 2. The current rMSSD results were not significant between

higher and lower levels HN. However, given that both rMSSD and HF are generally representative of parasympathetic activity, this could allude to a loss of parasympathetic cardiac rhythm modulation with decreasing HN, which is further supported by the increasing LF/HF.

Of interest are the RPA measures, particularly given the advantage in describing nonlinear, dynamical systems such as cardiac signals. A decrease in REC and DET and an increase in DIV measures are indicative of increased complexity and chaotic behavior of a time series [18]. Indeed, this is what was observed in the current study, with increasing HN levels associated with RPA measures reflective of more complex, short-term variability of the cardiac rhythm, implying a possible link with increased risk of cardiac arrhythmia.

The concept of mitohormesis could explain the current finding of HRV features showing convex or concave trend. Given the important role of ROS in various cellular functions [2], a biphasic mitohormesis model of human health response to mitochondrial OS has been suggested [19]. In addition, some level of mitochondrial ROS improves overall resistance to OS, which may be reflected in the associated changes in HRV features. Given the antioxidant properties of HN and the positive outcomes of studies utilizing humanin-analogues in improving OS, a cytoprotective effect on the heart [20] and effectiveness in Alzheimer's disease-related pathology [21], our study supports the hypothesis that humanin or its analogs may also be neuroprotective for the ANS.

To the best of our knowledge, the relationship between HN and HRV has not been described. However, an association between OS and autonomic modulation of cardiac rhythm has been reported in both healthy and disease populations. Given the important role of HN in lowering mitochondrial OS, our results could be interpreted as the impact of HN levels on autonomic modulation through its antioxidant properties. Future studies should investigate mitochondrial markers other than HN to gain insight into mitochondrial influence on the ANS through other MDPS such as MOTS-c, which potentially alleviates OS [22], and P66-Shc, an adaptor protein that promotes ROS production [23]. Furthermore, given that non-linear HRV measures showed the greatest differences, more non-linear measures could be explored in subsequent investigations to observe whether similar results are obtained. Finally, the presence of non-monotonic relationships between HN concentration and HRV parameters implies the necessity for measures beyond linear modelling to fully grasp its complexity.
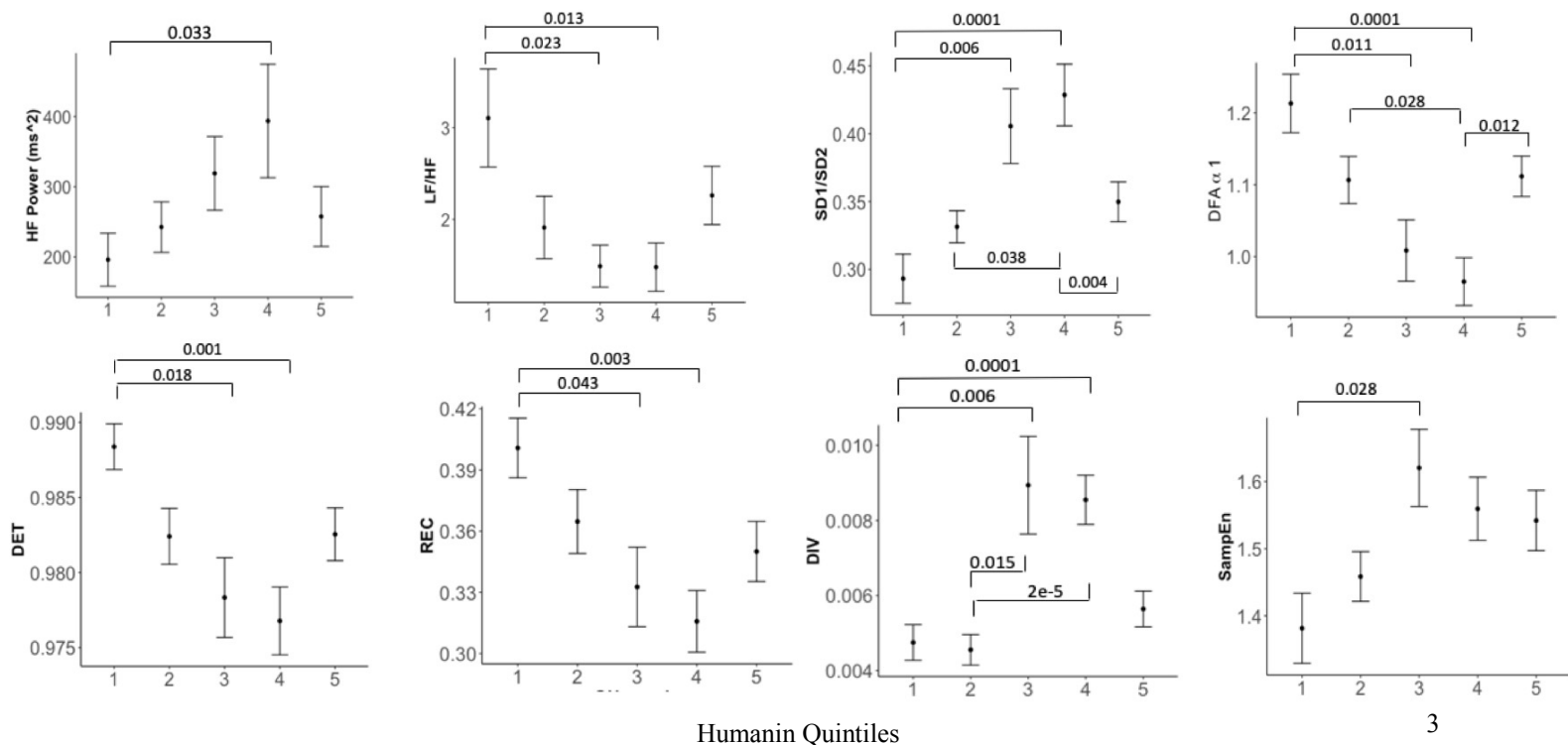
Acknowledgements

Figure 1: Means and standard errors of HRV measures across HN quintiles, along with the significant results of ANOVA and Games-Howell tests (p<0.05)

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