**Heart rate variability, interbeat interval, and their interrelationship in healthy subjects, HCN4
mutation carriers, and patients with anorexia nervosa**

Running title: Anorexia nervosa and HRV saturation

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Abstract

Objective: The purpose of this investigation was to examine heart rate variability (HRV), interbeat interval (IBI), and their interrelationship in healthy subjects, bradycardic HCN4 mutation carriers, and patients with anorexia nervosa (AN). We hypothesized that bradycardia in patients with AN is caused by neural mechanisms. Therefore, we assumed that a saturation effect of the HRV/IBI relationship in consequence of sustained parasympathetic control of the sinus node is exclusively detectable in patients with AN.

Method: Patients with AN between the ages of 12 and 16 years admitted to our hospital due to malnutrition were grouped (N = 20). A matched-pair group with healthy children and adolescents was created. Groups were matched for age and gender. We performed 24-hour Holter electrocardiography (ECG) in controls and patients. Specifically, all patients underwent two 24-hour Holter ECGs (admission; refeeding treatment). Additionally, IBI's were recorded during night-time in HCN4 mutation carriers (N = 4). HRV parameters were analyzed in 5-minute sequences during night-time and plotted against mean corresponding IBI length. HRV, IBI, and their interrelationship were examined by calculating saturation indices, Spearman's rank correlation analyses, Mann–Whitney U- and Wilcoxon signed-rank tests.

Results: The relationship between IBI and HRV showed signs of saturation in patients with AN. Signs of HRV saturation were also present in two HCN4 mutation carriers. In contrast, signs of HRV saturation were barely present in controls.

Discussion: We believe that non-neural mechanisms such as HCN4 down-regulation are possible explanations for HRV saturation and the associated bradycardia in patients with AN.

Keywords: Anorexia nervosa; autonomic nervous system; electrocardiography; heart rate

1. Introduction

In the last few years, it has increasingly been suggested that HRV is partly just a nonlinear surrogate for heart rate (HR) (M. Boyett, Wang, & D'Souza, 2019; M. R. Boyett et al., 2013). The studies conducted by D'Souza et al. imply that primarily alterations in the intrinsic HR via regulation of the ionic channels are responsible for HRV changes and bradycardia in athletes (A. D'Souza et al., 2014; Alicia D'Souza et al., 2017). Consequently, the theory of an increased vagal tone or a decreased sympathetic tone as a cause of starvation- or exercise-induced bradycardia has been questioned by some researchers (M. R. Boyett et al., 2013; Heathers, Nagata, & Murray, 2018; Leicht, 2013). Likewise, HRV metrics as an index of autonomic regulation have been challenged (M. Boyett et al., 2019; M. R. Boyett, 2017).

Considering these arguments, we analyzed HRV and interbeat interval (IBI) lengths in night-time ECG segments of patients with anorexia nervosa (AN) before and during refeeding therapy, patients with hyperpolarization-activated cyclic nucleotide-gated channel 4 (HCN4) mutation, and healthy controls. HRV metrics, their dynamics during refeeding therapy, and their relationship with IBI may offer new insights into the underlying mechanisms of bradycardia in patients with AN.

While non-neural mechanisms of bradycardia, such as HCN4 down-regulation or the HCN4 c.1737+1G>T mutation, presumably express themselves in HRV boost, a drug-induced parasympathetic dominance has shown to lead to a saturation effect of HRV metrics (de Geus, Gianaros, Brindle, Jennings, & Berntson; Goldberger, Challapalli, Tung, Parker, & Kadish, 2001; Kozasa et al., 2018). This association shows that HRV may constantly increase with declining HRs in HCN4 mutation carriers or subjects with HCN4 down-regulation, while HRV uncouples itself from HR changes at a certain point and then decreases with longer IBI's in subjects with a high vagal tone. The underlying mechanisms of HRV saturation are probably high-intensity vagal nerve discharges during expiration as part of respiratory sinus arrhythmia. If enough acetylcholine (ACh) is released during expiration, ACh concentrations in the sinus node area also remain high during inspiration, and the parasympathetic effect persists during the whole respiration cycle, resulting in a low HRV and low HR (figure 1) (de Geus et al.; Goldberger et al., 2001). Thus, our hypothesis suggested that HRV saturation occurs in patients with AN, while such an effect remains unverifiable in controls and patients with the HCN4 c.1737+1G>T

mutation, given that the observed bradycardia in patients with AN is caused by increased cardiovagal modulation and not by non-neural mechanisms such as HCN4 down-regulation.

2. Methods

All study participants provided informed consent, and the study design was approved by the appropriate ethics review board.

2.1 HRV

For an exhaustive explanation of all the common HRV metrics, their calculation, meaning, and clinical use, we referred to the study by Shaffer et al., Hayano et al. and the guidelines of the task force of the European society of cardiology and the North American society of pacing and electrophysiology (Hayano & Yuda, 2019; "Heart rate variability: standards of measurement, physiological interpretation and clinical use. Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology," 1996; F. Shaffer, McCraty, & Zerr, 2014).

2.2 Design

We examined the effects of nutritional refeeding on HRV parameters and HR in 20 adolescents with AN during in-hospital refeeding. To assess changes in HRV parameters, a 24-hour ECG was performed at admission and during nutritional refeeding. The second ECG was recorded between the 5th and 14th-day post admission when a high-calorie intake (>1500 kilocalories) and patient compliance were achieved. For our control group, we used a database containing 151 24-hour ECGs from healthy individuals aged 2–23 years (Bobkowski et al., 2017). Each patient was randomly matched to control by age (± 1 year) and gender. The monitoring period started between 07:00 and 12:00 and was discontinued on the second day. The Medilog Darwin ECG system (used holter recorder: medilog FD4 or medilog AR4, Schiller, Switzerland; sampling rate 250 Hz) was used for all ECG recordings of controls and patients with AN. An experienced cardiologist edited and inspected all ECG recordings for nonsinus beats or artifacts. The software Notepad++ was used to count beat annotations. ECGs with less than 95% sinus beats were excluded. For HCN4 mutation carriers, we used Polar® H10 chest belts to

record night-time RR intervals in an ambulant setting. Because of an unavailable ECG export function, 24-hour ECGs could not be used for our HRV calculations but we ruled out severe dysrhythmias in these patients.

Td- and fd parameters were calculated from exported 5-minute IBI segments with the Kubios HRV Premium 3.1.0 software for all patients with AN, controls, and patients with HCN4 mutation (Tarvainen, Niskanen, Lipponen, Ranta-aho, & Karjalainen, 2014). Nonsinus beats and artifacts were corrected by an automatic correction algorithm. The following td and fd parameters were determined: root mean square of successive differences (RMSSD), percentage of successive NN intervals that differ by more than 50 ms (pNN50), and high frequency (HF, 0.15–0.40 Hz) power in absolute units. HF power was calculated based on the Lomb–Scargle Periodograms. A smoothing factor of 0.02 Hz was applied.

2.3 Statistics

For the statistical analysis, we selected 5-minute sequences of recordings in sleeping patients and controls at night. The night-time sequences were identified by a slopping HR at night, by an increasing HR in the morning, and the time designation of our recordings. For the analysis, we excluded segments that showed >5% according to the Kubios software-corrected artifacts. All other segments which were recorded during sleep were part of the analyses. The fluctuations of HR and therefore, autonomic tone during different sleep stages were desired and enabled the evaluation of the IBI–HRV relationship in patients and controls. Median values of night-time HRV for each patient and control were calculated. Based on the median values, mean values were calculated for each group (table 1). Statistical analysis was performed on the SPSS version 25.0. The Shapiro–Wilk test was used to test whether the samples were obtained from a normal distribution. Therefore, the test showed that data distribution was partly non-Gaussian and that non-parametric tests were used. First, we compared IBIs and HRV parameters before and after nutritional refeeding using Wilcoxon signed-rank tests. Second, we compared IBI and HRV values of patients with AN to HRV and IBI values from controls using Mann–Whitney U tests. We considered $p < 0.01$ as statistically significant. Third, we examined the relationship between HF power and IBI using the Spearman’s rank correlation coefficient for each control, each patient with AN, and each patient with the HCN4 mutation. If Spearman’s rank correlation coefficient attained a negative

value, the relationship between IBI and HF power was defined as saturated (Kiviniemi et al., 2004). Fourth, IBI versus pNN50 values were plotted for each patient (patients with AN at admission and during refeeding), HCN4 mutation carrier, and control, and fitted to quadratic and linear models (supplementary material) (Kiviniemi et al., 2004). Fifth, saturation indices were calculated (supplementary material).

2.4 Patients

Twenty patients who met AN's Diagnostic and Statistical Manual of Mental Disorders V criteria were admitted to our hospital between June 2015 and December 2018 and included in the present analysis. Patients with severe depression, anxiety disorders, obsessive-compulsive disorders, psychotic disorders, sleep disorders, developmental disorders, and cardiovascular diseases in their medical history were excluded from the analysis. For all patients, parents consented to a 24-hour ECG. Additionally, all patients were supervised by our psychologist.

All data were retrospectively anonymized and analyzed. The analysis was approved by the university ethics committee (University of Würzburg No 143/16-mk). The treatment protocol was standardized. Physicians prescribed diets starting around 1200 kcal per day, and then increased by at least 200 kcal/day up to a normal caloric intake between 2500 and 3000 kcal. Targeted caloric intake was achieved by supplementing most patients with high-energy liquid nutrition, starting with 10 kcal/kg/day. Nasogastric tube feeding was not required. Patients were supervised during meals and snack, for up to 30 minutes post-consumption by room sitters, mostly nurses. Patients' weight was measured each morning with the same scale under standardized conditions (i.e., consistent clothing, weighing before the patient visited the bathroom). Fluid balance, blood count, and serum electrolytes were measured at admission and later, according to the doctor's orders. Nutritional supplements were routinely administered (i.e., omega-3-fatty acids, calcium glycerol phosphate, zinc gluconate, magnesium, b-vitamins, vitamin C, and vitamin K). Controlled exercise phases were allowed. Outdoor walking or running, gymnastics, and physiotherapy were offered to patients. Following sufficient weight gain and clinical stabilization, all patients were either referred to a psychotherapeutic clinic specializing in eating disorders or supervised in an outpatient setting.

Further, 3 female patients and one male patient carrying the HCN4 c.1737+1G>T mutation consented to night-time IBI recordings and were included in our study (Age: 13, 23, 27, and 22 years). Prof. Dr. Robert Sepp recruited these patients from a former study (Hategan et al., 2017). The 4 patients did not take any drugs during IBI recording, nor were they athletes. Molecular genetic analyses and ECG recordings were approved by the Institutional Ethics Committee of the University of Szeged, Hungary.

3. Results

Twenty patients with AN (18 women) were enrolled with a mean BMI percentile of 2.59 (standard deviation: 6.17) at admission and a mean BMI percentile of 5.92 (standard deviation: 11.30) at hospital release. Table 1 summarizes patient characteristics. Following are relevant characteristics not present in Table 1: Seven patients had low triiodothyronine levels, six patients had hypermagnesemia, two patients had anemia, one patient had hyponatremia, one patient had leukopenia, and one patient had a mild pericardial effusion. Furthermore, one patient had postural orthostatic tachycardia syndrome and tested positive for anti-alpha-adrenergic antibodies.

3.1 HRV and IBI during starvation and refeeding

Table 1 summarizes HRV parameters and IBIs of patients and controls. Upon admission, HRV parameters of patients with AN were high, and HRs were low compared with controls (pNN50: +117.28%, RMSSD: +103.42%, HF power: +383.27%, IBI: + 36.31%).

During refeeding, we observed increasing HRs in 20 patients and varying changes in HRV metrics. In 11 patients, pNN50 and RMSSD increased, while in 9 patients, pNN50 and RMSSD decreased. HF power decreased in 8 patients, while in 12 patients, HF power increased.

3.2 HRV and IBI in patients with HCN4 mutation

Table 1 shows HRV parameters and IBIs of patients with HCN4 mutation. Compared with controls, HRV and IBI values were high in patients with HCN4 mutation (pNN50: +121.45%, RMSSD: +122.23%, HF power: +357.51%, IBI: +37.49%).

3.3 Relationship between IBI and HRV metrics

We observed a positive correlation between IBI and HF power in 19 of 20 controls (mean Spearman's rho rank-order correlation coefficient: 0.52). In contrast, patients with AN had either a weak positive correlation between HF power and IBI or a negative Spearman's correlation coefficient at admission (mean Spearman's rho rank-order correlation coefficient: 0.05). During refeeding, the correlation between HF power and IBI strengthened in many patients, approximating normal values (Table 2). In two of four HCN4 mutation carriers, we observed a positive correlation between HF power and IBI (rank-order correlation coefficient: 0.49 and 0.50). However, in two patients we observed a negative correlation between HF power and IBI (rank-order correlation coefficient: -0.01 and -0.22).

In all patients and controls, the quadratic function was superior or equally fitting to a linear function in its ability to describe the relationship between IBI and pNN50 (supplementary material). Average saturation indices were higher in patients with AN and HCN4 mutation carriers than in controls (patients with AN at admission: 20.50%; patients with AN during refeeding: 11.73%; HCN4 mutation carriers: 16.61%; controls: 6.25%). However, saturation indices varied to a large extent between individuals (supplementary material).

4. Discussion

This investigation demonstrated that the relationship between IBI and HRV showed signs of saturation in patients with AN and in two patients with the HCN4 c.1737+1G>T mutation. In contrast, signs of HRV saturation were barely present in controls. Additionally, a significant increase in HR during nutritional refeeding was observed, whereas HRV parameters either increased or decreased.

4.1 HRV and HR changes during starvation and refeeding

Our findings are highly consistent with studies measuring HRV before nutritional refeeding in acute AN (Galetta et al., 2003; Petretta et al., 1997). Earlier studies found increased HRV values in patients with AN (Mazurak, Enck, Muth, Teufel, & Zipfel, 2011). Upon admission and before nutritional refeeding, our analysis revealed the presence of sinus bradycardia and signs of vagus activation. RMSSD, which

is the primary parameter used to estimate the parasympathetically mediated changes reflected in HRV, was significantly increased in patients with AN (table 1).

After high-calorie refeeding, mean IBI values of controls were approximated, while HRV parameters either increased or decreased (table 1). The significantly increasing HR during refeeding treatment suggests that bradycardia is an adaptive response to malnutrition in patients with AN (Galetta et al., 2003). Bradycardia presumably decreases energy consumption and is effective in regulating emotional stress (Park & Thayer, 2014).

4.2 Relationship between IBI and HRV

Recent studies suggest that HRV is partly a nonlinear surrogate for HR (Monfredi et al., 2014; Rocchetti, Malfatto, Lombardi, & Zaza, 2000; Zaza & Lombardi, 2001). Therefore, it was asserted that HRV parameters increase fundamentally with decreasing HRs and increasing IBIs due to mathematical and physiological reasons (M. Boyett et al., 2019; M. R. Boyett, 2017; M. R. Boyett et al., 2017; Gąsior, Sacha, Jeleń, Zieliński, & Przybylski, 2016; Monfredi et al., 2014; Sacha, 2013). Boyett et al. concluded that high HR levels with steep pacemaker potentials produce relatively (little variability between successive heart beats) small changes in the IBI, whereas low HR levels with flat pacemaker potentials produce relatively large changes in the IBI (M. Boyett et al., 2019). Consequently, HRV parameters were corrected for the measured chronotropic state (Gąsior et al., 2018; Sacha, 2013; van den Berg et al., 2018). Here, we demonstrate that HRV is not simply a surrogate for HR or IBI in patients with AN. We observed dissociation between HRV parameters and IBI in patients with AN (supplementary material and table 2).

We calculated Spearman's correlation coefficients for the IBI–HF power relationship for each patient and control. The fd parameter of HF power has been shown to quantify the magnitude of respiratory sinus arrhythmia (Berntson, Cacioppo, & Quigley, 1993). Moreover, HF power has been widely used as an index of parasympathetic activity in various studies (Fred Shaffer & Ginsberg, 2017). The correlation between IBI and HF power was much weaker in patients with AN than in controls. In 8 of 20 patients with AN, we even found a negative Spearman's correlation coefficient. A Spearman's

correlation coefficient lesser than zero implies that most HF power values and corresponding IBIs of a patient are likely located at the descending limb of a quadratic HRV–IBI function. Consequently, HRV saturation was present in at least 8 patients with AN at admission and at least 7 patients with AN during refeeding treatment (table 2).

The fact that Spearman’s correlation coefficients were similar with controls in some patients with AN may be due to individually different IBI–HF power relationships. Kiviniemi et al. showed that the deflection point of a quadratic IBI–HF power relationship in adults can be found within a range of 1010–1788 ms (Kiviniemi et al., 2004). Further, the period in which a distinct HRV saturation is detectable may be restricted to severe starvation phases, which some patients may not have experienced. Evidence of this may be that signs of HRV saturation partly disappeared during refeeding treatment (table 2 and supplementary material). During nutritional refeeding, Spearman’s correlation coefficients increased in 11 of 20 patients implying a shift toward the ascending limb of the curve (table 2).

In patients with the HCN4 c.1737+1G>T mutation, our HRV and IBI analysis revealed contradictory results. In 2 patients, a strong correlation between IBI and HF power was present, which confirmed the theory of HRV boost in HCN4 mutation carriers (de Geus et al.). As part of HRV boost, HRV may have constantly increased with decreasing HRs due to the absence of a parasympathetically mediated HRV saturation effect. Because of the HCN4 mutation the funny channel may have had no “limiter” function for the bradycardic effects induced by vagal activity (de Geus et al.).

On the other hand, two patients had a negative Spearman’s correlation coefficient, which implied the presence of HRV saturation. Also, the pNN50/IBI graphs indicated HRV saturation in these patients (figure 2 and supplementary material). Possible explanations for the contradictory results are diverse and comprise individually different IBI–HRV relationships or an exercise-induced high parasympathetic tone in patients with signs of HRV saturation. Moreover, it is possible that the 23- and 22-year-old patient were not bradycardic enough for HRV saturation (supplementary material) (Goldberger et al., 2001).

4.3 Neural or non-neural mechanisms?

In athletes, a decrease in HRV at very low HRs has been observed (Plews, Laursen, Stanley, Kilding, & Buchheit, 2013). Considering the potential overlap between AN and athletics, it is not surprising that similar HRV/IBI dynamics can be observed in athletes. Endurance-based exercise patterns are common in AN. Excessive exercise can be regarded as a compensatory behavior, aimed at losing weight. Therefore, the combination of athletic bradycardia and starvation may contribute to bradycardia in AN. It is worth noting that patients with AN and athletes showed similar HRV–IBI dynamics as subjects who successively underwent sympathetic blockade, parasympathetic stimulation, and parasympathetic blockade (Goldberger et al., 2001). The healthy subjects who successively underwent sympathetic blockade, parasympathetic stimulation, and parasympathetic blockade also showed an HRV saturation effect. Goldberger has suggested that the underlying mechanism of an HRV saturation effect is a sustained parasympathetic control of the sinus node, which eliminates respiratory heart modulation and reduces HRV (figure 1) (Goldberger et al., 2001). This effect is likely because the parasympathetic impact on IBI is mediated via release of the neurotransmitter ACh by the vagus nerve and M2-muscarinic ACh receptors. Downstream changes to ionic currents and cell membrane conductance initiate a slower diastolic depolarization. During each breathing cycle, a parasympathetic-mediated variation of HR naturally occurs. This phenomenon is called respiratory sinus arrhythmia. During inspiration, activated baroreceptors diminish the vagal tone via the central autonomic network which is followed by increase in HR. In contrast, during expiration, baroreceptors are less active, and HR, therefore, decreases (Berntson et al., 1993). These fluctuations are one of the several other factors that cause HRV. In patients with AN, these fluctuations in the breathing cycle may attenuate at low HRs during certain sleep stages with a high parasympathetic influence. During expiration, the vagus nerve possibly releases high amounts of ACh, which cannot be sufficiently cleaved by low acetylcholinesterase levels (Chamberlain, Turner, & Sneddon, 1967; Dewland, Androne, Lee, Lampert, & Katz, 2007). As a result, the expected decline in the concentration of ACh during inspiration in the sinus node area may be absent, and high concentrations of ACh persist during inspiration and expiration. This leads to sustained parasympathetic control of the sinus node during the whole breathing cycle, which eliminates respiratory heart modulation and, therefore, reduces HRV at very low HRs (Goldberger et al., 2001).

To our surprise, we could find signs of HRV saturation in two HCN4 mutation carriers (supplementary material and section 4.2.). This finding implies that a sustained parasympathetic control of the sinus node may not be the only explanation for HRV saturation and associated bradycardia in patients with AN. Our observation of similar HRV dynamics in HCN4 mutation carriers and patients with AN supports the theory of Boyett et al. who have suggested that non-neural mechanisms such as HCN4 down-regulation are responsible for bradycardia in athletes (A. D'Souza et al., 2014).

In patients with the HCN4 c.1737+1G>T mutation, bradycardia is likely attributable to the consequences of the present mutation and not to a high parasympathetic tone. The HCN4 c.1737+1G>T mutation supposedly leads to conformational changes in the C-linker part of the HCN4 protein, very close to the cyclic adenosine monophosphate binding domain. Therefore, the mutation likely affects cAMP binding. The HCN4 c.1737+1G>T mutation possibly makes HCN4 less sensitive to cAMP, which leads to an decrease of the steepness of diastolic depolarization and creates an imbalance between the I_f current and the counteracting parasympathetically activated I_{KACH} current (Gordan, Gwathmey, & Xie, 2015). Reduced cAMP sensitivity and low cAMP levels cause a polarizing shift in the voltage dependence of the I_f activation curve, meaning that less I_f current is available at diastolic potentials (Monfredi, Maltsev, & Lakatta, 2013).

Consequently, diastolic depolarization time is prolonged, and HR slows down. However, this effect cannot explain HRV saturation. One possible theory is as follows: If cAMP insensitivity and low cAMP levels drastically prolong diastolic depolarization via I_f , other rhythmic intrinsic pacemaker mechanisms, that may be less influenced by autonomic tone than the funny channel become dominant and intervene to prevent sinus arrest (Ardell, Randall, Pomeroy, Lawton, & Kim, 1991; Lakatta Edward, Maltsev Victor, & Vinogradova Tatiana, 2010; Monfredi et al., 2013; Sealy, Bache, Seaber, & Bhattacharga, 1973). This would explain the phenomenon of a low HRV at long RR intervals.

4.4 Implications

The relationship between IBI and HRV in patients with AN has three consequences. First, common explanations of HRV saturation may be fundamentally flawed. Therefore, HRV saturation may not prove a parasympathetically induced bradycardia. Second, night-time HRV parameters measured in a

supine position may only be partly suitable to assess the vagal outflow because the saturation effect may underestimate parasympathetic activity at long IBI levels. Third, HRV correction formulas should be used with caution in patients with bradycardia. Subject-specific correction formulas may perform better than standard ones (Malik, Farbom, Batchvarov, Hnatkova, & Camm, 2002).

4.5 Clinical use of HRV and HR monitoring

We demonstrated that during refeeding therapy, HR increased in 20 patients. Therefore, suspected starvation in patients with AN can be confirmed by the observation of decreasing night-time HRs. Additionally, HR monitoring could be used to maximize the likelihood of detecting recidivism in an ambulant setting and facilitate a timely intervention to avoid severe somatic consequences. In contrast to weight controls, night-time HRs are hard to manipulate and represent a better parameter to assess treatment success, especially in the absence of standardized weight controls.

In our study, we showed that HRV carries other information than HR. Due to the saturation effect, night-time HRV monitoring may be inferior to HR monitoring to assess the treatment success.

4.6 Limitations

The sample size of this investigation was relatively small, especially the number of patients with HCN4 mutations. The implication that mechanisms other than autonomic regulation may be responsible for HRV saturation and the associated bradycardia in patients with AN were based on the data of two patients. Moreover, the other 2 patients showed the expected HRV boosts, which supports the theory of increased cardiovagal modulation in patients with AN (supplementary material). For future studies, larger sample sizes are required. Second, we cannot state with certainty whether the HCN4 c.1737+1G>T mutation has similar effects as HCN4 down-regulation. Third, undetected or unconsidered comorbidities may have influenced our results. Because patients with AN often carry several comorbidities, we believe that the resulting HRV and HR values are affected by these various comorbid syndromes. Results from several earlier studies confirm the hypothesis that depression can lower HRV or that autonomic dysfunction can contribute to the development of depression (Agelink, Boz, Ullrich, & Andrich, 2002; Huang et al., 2018). Additionally, antidepressants and antipsychotic

drugs can lower HRV (Licht, de Geus, van Dyck, & Penninx, 2009). However, in this study, patients with major comorbidities were excluded (see section 2), antidepressants were prescribed with caution, and selective serotonin reuptake inhibitors were preferred (Kemp et al., 2010; O'Regan, Kenny, Cronin, Finucane, & Kearney, 2015; van Zyl, Hasegawa, & Nagata, 2008). Fourth, confounders such as respiration, daytime exercise level, blood pressure fluctuation or sleep stages were not controlled. Also, patients with AN were not tested for genetic conditions that could have caused bradycardia. Fifth, we only analyzed two Holter ECGs of each patient, one before and one during refeeding. For future studies examining treatment effects, we recommend calculating two- or three-day HRV average values. Also, the administration of omega-3 fatty acids and other nutritional supplements may have influenced HRV and HR. Finally, electrolyte abnormalities, altered hormone levels could have been responsible for changes in HRV and HR. We also assumed that the HRV–IBI relationship is quadratic with an ascending and descending limb. However, it is likely that the HRV–IBI relationship is different between individuals and cannot be described using a quadratic function for every subject. Goodness-of-fit metrics, such as R^2 , varied to a large extent between patients. In some patients, very low R^2 values were observed (supplementary material).

5. Conclusions

Our hypothesis, which states that bradycardia in patients with AN is caused by neural mechanisms, could not be verified. HRV saturation occurs in patients with AN; however, signs of HRV saturation were also present in HCN4 mutation carriers. Therefore, the theory of increased cardiovagal modulation as a cause of bradycardia in patients with AN may be unsatisfactory. Metabolic rate and excessive exercise may influence heart rate via HCN4 regulation. Knowledge of the mechanisms of bradycardia and HRV in patients with AN may lead to the development of novel target therapies in the future.

Data Availability: The datasets analyzed in this study are available from Reiner Buchhorn or Christoph Baumann on reasonable request.

Preprint: <https://psyarxiv.com/qpgz4/>

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7. Tables

Table 1. Patient characteristics and HRV metrics

		Age (years)	Disease duration (in months)	Calorie intake (in kilocalories)	Night-time mean IBI (ms)	RMSSD (ms)	pNN50 (in %)	HF (ms ²)
Healthy controls		14.00 ±	-	-	885.98 ±	55.86 ±	28.01 ±	1250.32 ±
		1.26			136.54***	23.84***	18.26***	934.11***
Patients with HCN4 mutation		21.25 ±	-	—	1218.09 ±	124.14 ± 25.83	62.03 ±	5720.33 ± 2341.28
		5.91			230.93		8.39	
Patients with anorexia nervosa	At admission	14.00 ±	8.92 ± 6.70	829.09 ± 416.30	1391.15 ±	113.63 ±	60.86 ±	6277.07 ±
		1.34			283.94****	48.28*	12.87*	6172.19*
	During	-	—	2641.18 ±	1075.91 ±	107.90 ±	54.65 ±	5060.45 ±
	refeeding			416.30	165.41*****	45.27**	17.06**	3810.14**

Data shown represent the mean ± standard deviation. Significance of difference: Controls vs. patients with AN at admission -> * = ($p < 0.01$); controls vs. patients with AN at release -> ** = ($p < 0.01$); patients with AN at admission vs. patients with AN at release -> *** = ($p < 0.01$).

Table 2. Spearman's correlation coefficients

Patients with anorexia nervosa						Controls			
Patient number	Age (years)	Number of samples at admission (ECG-5- minute- segments)	HF-IBI correlation at admission	Number of samples during refeeding (ECG-5- minute- segments)	HF-IBI correlation during refeeding	Control number	Age (years)	Number of samples (ECG- 5-minute- segments)	HF-IBI correlation
1	12	91	0.307	88	0.719	1	12	88	0.819
2	13	135	0.909	113	0.526	2	13	113	-0.248
3	14	104	-0.434	100	-0.035	3	14	100	0.755
4	14	23	-0.509	86	-0.381	4	14	86	0.679
5	13	97	-0.212	92	0.196	5	13	92	0.381
6	16	100	-0.606	70	-0.532	6	16	70	0.493
7	13	114	0.07	96	-0.497	7	13	96	0.785

8	13	94	0.661	102	0.347	8	13	102	0.137
9	13	104	−0.557	44	0.677	9	13	44	0.473
10	14	114	0.273	98	−0.066	10	14	98	0.81
11	12	96	0.641	106	0.274	11	12	106	0.669
12	15	95	0.345	93	0.31	12	15	93	0.69
13	16	104	−0.246	38	0.813	13	16	38	0.79
14	14	68	−0.905	85	0.746	14	14	85	0.052
15	16	111	0.783	108	0.265	15	16	108	0.685
16	15	68	−0.33	102	0.515	16	15	102	0.383
17	16	114	0.176	101	−0.24	17	15	101	0.775
18	15	124	0.325	37	−0.336	18	15	37	0.364
19	13	86	0.196	88	0.888	19	13	88	0.918
20	13	32	0.044	95	0.809	20	14	100	0.600

Negative
Spearman's
correlation
coefficient
87
1

Significance of differences: bold numbers = $p < 0.01$

8. Figures

Figure 1. Respiration-related episodic acetylcholine release (HRV saturation on the left side, no HRV saturation on the right side). On the left side, ACh levels remain high during inspiration and expiration. Consequently, the IBI difference between inspiration and expiration is lesser in patients with an HRV saturation effect than in subjects without an HRV saturation effect (50 ms vs. 100 ms). This phenomenon then leads to low HRV values at long IBIs (de Geus et al.). From: de Geus; Should heart rate variability be “corrected” for heart rate? Biological, quantitative, and interpretive considerations; CC BY 4.0; www.onlinelibrary.wiley.com/doi/full/10.1111/psyp.13287; adapted version.

Figure 2. Relationship between pNN50 and IBI in a 27-year-old patient carrying the HCN4 c.1737+1G>T mutation. A distinct HRV saturation is visible. (Quadratic function: $pNN50 = 1.154 \times IBI^2 - 0.001 \times IBI - 589.530$ $R^2 = .197$; linear function: $pNN50 = -0.004 \times IBI + 62.062$ $R^2 = .000$; saturation index: 34.31%).