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Spectral and Nonlinear Analysis of Electrodermal Activity in Adolescent Anorexia Nervosa

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Abstract: Anorexia nervosa (AN) is an eating disorder with increasing prevalence in childhood and adolescence. Sympathetic dysregulation is supposed to be the underlying mechanism of increased cardiovascular risk in AN. Thus, we assess the electrodermal activity (EDA) as a non-invasive index of sympathetic cholinergic activity using linear and nonlinear analysis in adolescent AN with the aim of detecting potential biomarkers for AN-linked cardiovascular risk. We examined 25 adolescent girls with AN and 25 age-matched controls. EDA was continuously recorded during a 5-min resting phase. Evaluated parameters were: time-domain (skin conductance level, non-specific skin conductance responses), frequency-domain (EDA in very low, low, sympathetic, high and very high frequency bands) and nonlinear (approximate, sample, symbolic information entropies, detrended fluctuation analysis (DFA)) parameters of EDA and peripheral skin temperature. Our findings revealed lower EDA values indicating a decrease in the sympathetic nervous activity in female adolescents with the acute phase of AN. Further, we found higher nonlinear index DFA in AN vs. controls. We assumed that nonlinear index DFA could provide novel and independent information on the complex sympathetic regulatory network. We conclude that the parameters of complex EDA analysis could be used as sensitive biomarkers for the assessment of sympathetic cholinergic dysregulation as a risk factor for AN-linked cardiovascular morbidity.

Keywords: anorexia nervosa; electrodermal activity; time-domain analysis; frequency-domain analysis; nonlinear analysis

1. Introduction

Anorexia nervosa (AN) is one of the most frequent eating disorders with the highest morbidity and mortality among intake malfunctions [1,2]. Patients with AN are characterised by low weight as a result of the restriction of food intake, fear of weight gain, and distortion of self-image, including fear of being or becoming fat [3,4]. Rates of lifetime prevalence of AN range from 1.4% to 3.3% in

women [5]. AN mainly presents in mid- to late adolescence, although its occurrence in childhood and early adolescence is increasing [6].

In general, the autonomic nervous system (ANS) plays a crucial role in the maintenance of the homeostasis, flexibility and adaptability of the human body. It is assumed that disturbances of the ANS may participate in underlying mechanisms of cardiovascular disorders associated with AN [7]. For example, several studies revealed lower heart rate associated with higher heart rate variability indicating cardiovagal dominance, and sympathetic vascular underactivity indexed by lower blood pressure variability [7–11]. However, studies regarding other biosignals in AN are scarce.

Electrodermal activity (EDA) represents a non-invasive index of sympathetic cholinergic activity. Specifically, changes in sympathetic activity lead to altered activity of eccrine sweat glands, which is related to skin´s ability to conduct electricity and thus to EDA variations [12,13]. Moreover, the eccrine sweat glands are involved in thermoregulation of organisms, and cover the majority of the organism, but are the densest on palms and soles. Indeed, these localizations are mainly used in psychophysiological research, because it is assumed that they are associated with emotion-evoked sweating [13].

From the physiological aspect, the skin is relatively non-conductive, due to its most upper layer of derma—the *stratum corneum*. A variable amount of sweat is released from the ducts of the eccrine sweat glands, which open onto the skin surface. The degree of sympathetic activation affects the amount of filled sweat ducts, and the amount of produced sweat; in turn, this activation influences the degree of skin conduction. Variations of the amount of sweat in the ducts relates to the conductivity of the skin and its measurable changes in EDA [14]. Additionally, Posada-Quintero et al. (2016) revealed that variation in the dynamics of the sympathetic activity evaluated by EDA represent one of the important prognostic and diagnostic tools in diseases associated with autonomic dysfunction [15].

With respect to methodology, Lanata et al. (2012) evaluated changes of EDA by standard time and frequency indices and nonlinear parameters, such as deterministic chaos, recurrence plot, and detrended fluctuation analysis in the healthy group during exposure of sets of images from the International Affective Picture System (IAPS) [16]. This study revealed that nonlinear indices of EDA contribute to better extraction of EDA features, such as characteristics of sympathetic activity without significant differences in two types of used measuring devices (special textile gloves vs. bipolar electrodes) [16]. Our previous study evaluated the difference between standard and nonlinear parameters of EDA (approximate and symbolic information entropy) after cognitive stressors (recovery phase) in healthy young adults. Specifically, whereas skin conductance amplitude remained increased in the recovery phase compared to the baseline period, nonlinear indices decreased under the baseline value. It is assumed that this could be caused by complex mechanisms consisting of feedback loops forming a chaotic system on the central level [17].

Recently, evaluation of EDA in AN has been attracting more attention from scientists. Several studies found a lower amplitude of skin conductance response (SCR) in adult females suffering from AN versus age-matched healthy control (HC) in resting conditions [18–20]. Similarly, Crifaci et al. (2013) revealed a decrease of SCR, but a higher level of frequency of SCR in AN group with respect to healthy volunteers during baseline [10]. Additionally, Soussignan et al. (2011) observed significantly larger SCR during exposure to pictures of food and odorous foods in HC versus AN women [21]. Finally, AN women had lower SCR during rest and the Iowa Gambling Task compared to the HC group [22]. According to these results, Tchanturia et al. (2007) assumed that SCR represents a sensitive indicator of response to stimuli in AN patients [22]. While the variations of SCR in adult AN patients are relatively obvious [18,20], the changes of spectral components and nonlinear features of EDA in adolescent females suffering from AN remain unclear. Moreover, studies evaluating variables of EDA by time, spectral and nonlinear indices in AN adolescents are rare. There exist only a few studies concerning changes of these parameters, and only in healthy adults [15–17,23,24]. For example, time and spectral indices of EDA for frequency bands (range from 0.0 Hz to 0.5 Hz) increased during applications of various stressors (changes of position, cold pressor, and Stroop test) assuming that the

EDA evaluation by different parameters could provide an important quantitative tool for evaluating sudomotor activity regulated by the sympathetic nervous system [15].

Based on these studies, we hypothesized that complex EDA analysis (time-domain, spectral and non-linear) could provide important information related to sympathetic nervous activity in adolescent anorexic patients. Thus, we aimed to evaluate EDA by different parameters of the spectral and nonlinear analysis indicating complex sympathetic cholinergic regulatory network in adolescent anorexia nervosa. To the best of our knowledge, this is the first study to use complex EDA analysis with the aim of detecting potential biomarkers for complex sympathetic cholinergic dysregulation as a risk factor for AN-linked cardiovascular morbidity already present in adolescent age.

2. Materials and Methods

The study was approved by the Ethics Committee of Jessenius Faculty of Medicine in Martin, Comenius University in Bratislava in accordance with the 1964 Helsinki declaration and its later amendments. All participants and their parents/legal representatives were carefully instructed about the study protocol and gave written informed consent to participate in the study before the examination.

2.1. Study Population

The studied group consisted of 25 girls suffering from newly diagnosed AN recruited from inpatients admitted to the Psychiatric Clinic of Jessenius Faculty of Medicine and University Hospital in Martin (mean age: 14.8 ± 0.4 years), and control group of 25 healthy age-matched controls (mean age: 15.1 ± 0.3 years). Exclusion criteria were the following for both groups: history of recent acute illness or chronic cardiovascular, respiratory, endocrine, neurological, metabolic, and infectious diseases, smoking, medication/psychoactive substances, or dietary supplementation potentially affecting the ANS. Moreover, mental disorders and weight abnormalities (underweight/overweight/obesity) were considered as exclusion criteria in control group. The weight was defined according to extended International Obesity Task Force body mass index (BMI) cut-offs for thinness, overweight, and obesity using age- and sex- specific BMI cut-offs, which correspond to the adult BMI range between 18.5 and 25 kg/m^2 for normal weight, and the threshold of 30 kg/m^2 for obesity [25].

2.2. Diagnosis of Anorexia Nervosa

The diagnosis of anorexia nervosa without other comorbid mental disorders (e.g., depressive disorder) was assessed by a thorough clinical investigation based on a unstructured diagnostic interview by a staff child/adolescent psychiatrist according to the Diagnostic and Statistical Manual of Mental Disorders, DSM-5 (American Psychiatric Association, 2013). The AN patients were examined before pharmacotherapy and starting of nonpharmacological regimen interventions, including re-feeding, during the first days of hospitalization.

2.3. The Study Protocol and Evaluated Parameters

The examinations were performed in the Psychophysiological laboratory (Biomedical Center Martin, Jessenius Faculty of Medicine in Martin) under standard conditions with the minimalization of stimuli: a quiet room, 22 °C, in the morning between 8:00 and 11:30 a.m., after a normal breakfast.

Firstly, anthropometric parameters were assessed by body composition analyser InBody 120 (Biospace Co. Ltd, Seoul, Korea). Then, participants were instructed to sit comfortably and rest in a special armchair for 10 min to avoid potential effects of stress (laboratory environment and examining person). After the introductory 10 min, EDA was recorded using FlexComp Infinity Biofeedback (Thought Technology, Canada) with a sampling frequency of 256 Hz. The EDA signal was monitored by two bipolar electrodes composed of 10-mm diameter Ag-AgCl placed on the middle phalanges of two fingers on the non-dominant hand. In accordance with some other studies [26,27], the special EDA conductive gel was not used. Special FlexComp biofeedback electrodes containing a metallic disk set were used without conductive gel as recommended by Thought Technology, Ltd. [28]. These

special electrodes were carefully cleaned with an alcohol wipe after each examination of the subject. In addition, peripheral skin temperature was recorded using FlexComp Infinity Biofeedback (Thought Technology, Canada). The temperature of the periphery was monitored by a sensor attached to the second phalange of one finger on the non-dominant hand. The baseline phase of the study lasted for 5 min.

2.4. Anthropometric Measures

Body composition was analysed using InBody 120 (Biospace Co. Ltd, Seoul, Korea) with multi-segmental multi-frequency (20/100 kHz) bioimpedance analysis, using eight tactile electrodes (thumbs and fingers of the hands, balls of the feet and heels) in a standing position. Thereafter, software Lookin'Body120 (Biospace Co. Ltd, Korea) processed the obtained data. The following body composition parameters were evaluated: weight (kg) and body mass index (BMI, kg/m^2) calculated as the weight (kg) divided by the square of height (m), waist circumference (WC, cm). Body surface area (BSA, m^2) was calculated using the Mosteller equation as:

$$BSA = \sqrt{h \times w/3600}, \quad (1)$$

where h is height (cm) and w is weight (kg) [29].

3. Data Analysis

3.1. Time-Domain Parameters of EDA

The tonic EDA, needed for analysis, was extracted by 10th order low-pass finite impulse response filter (cut off 0.0004 Hz) [15]. Next, the skin conductance level (SCL) was evaluated as a mean amplitude of tonic EDA. SCL informs about quantitative changes in the cholinergic sympathetic nervous system. The physiological values of SCL depend on the size of the sensors used. For 10 mm sensors, the range is from 0 to 30 micro Siemens [14,30].

Non-specific skin conductance responses (NS.SCRs) were obtained as the rate of spontaneous skin conductance responses that occur without external stimuli during examination [31]. Typical values ranged from 1 to 3 per min during baseline. The higher values of NS.SCRs indicate intensified arousal conditions [14]. Thus, the time-domain parameters provide information about changes associated with the sympathetic nervous system [15].

3.2. Power Spectral Analysis of EDA

At first, the data was filtered with an 8th-order Chebyshev Type I low-pass filter. Consequently, the signals were down-sampled to 2 Hz. Then, to eliminate any trend the data was high-pass filtered with an 8th-order Butterworth filter. The power spectra of signals were evaluated using Welch´s periodogram method with a 50% overlap. The mean power spectrum of the analysed unit was computed by fast Fourier transform (using a Blackman window length of 128 samples), and spectral powers [μS^2] in the appropriate frequency bands (VLF: 0.000–0.045 Hz; EDA-Symp: 0.045–0.25 Hz (sum of low (0.045–0.15 Hz) and high 1 (0.15–0.25 Hz) frequencies); HF: 0.25–0.40 Hz and VHF: 0.40–0.50 Hz) were obtained according to Posada-Quintero et al. [15].

From a physiological perspective, the frequency-domain parameters express the spectral distribution of sympathetic arousal in the skin surface [15].

3.3. Nonlinear Indices of EDA

3.3.1. Entropy

Entropy describes the unpredictability, randomness and uncertainty of a system. Entropy represents random and asymmetrical fluctuations in a given time series, and thus the complexity of

the sympathetic cholinergic regulatory system [32]. The fundament of entropy indices calculation is the evaluation of probabilities distribution of the expected value in the time series [32,33].

3.3.2. Approximate and Sample Entropy of EDA

Approximate (ApEn) and Sample (SampEn) entropies describe the probability that the vectors of length m chosen from a time-series of length N that are related within a given tolerance range r, remain similar for the vector of length m + 1.

$$\text{ApEn}\ (m, r, N) = \Phi^m(r) - \Phi^{m+1}(r), \tag{2}$$

$$\text{SampEn}\ (m, r, N) = \log\left(\Phi^m(r) / \Phi^{m+1}(r)\right), \tag{3}$$

where N—number of points, m—length of sequence (for N = 300 length of sequence m = 2), r—tolerance of similarity (r ϵ< 0.1 SD; 0.25 SD>). Minimum values of ApEn and SampEn (around zero) indicate regular system. In contrast, the increasing ApEn and SampEn refer to a random and more complex system [32,34].

3.3.3. Symbolic Information Entropy

The analysis of symbolic information entropy (SIE) has been demonstrated to be sufficient for the investigation of complex systems and is able to describe nonlinear aspects within a time series. The concept of SIE is based on coarse-graining of the time series into symbolic sequences with a certainly given alphabet. This data was transformed by four numbers (0, 1, 2, 3) to classify the dynamic changes of the time series, then these symbols were used to generate SIE [17,24]. The numbers characterize changes of the time series, i.e., 0 and 1 indicate slow and fast increasing of the data, respectively; 2 and 3 specify fast and slowly decreasing signal, respectively. The patterns are divided into vectors of length L = 2 and they are paired into the class according to letters of the vector. For instance, vector 13 means the change of SCL from fast elevation to a slow decrease in the waveform. Index SIE characterizes the randomness and messiness of the system. Symbolic information entropy can be defined as

$$\text{SIE}(m) = -\Sigma^{M}{}_{i=1}\ (p_i * P_i), \tag{4}$$

where if $p_i > 0$, then $P_i = \log_2(p_i)$, else $P_i = 0$. The higher values of SIE represent more irregular, messy and unpredictable signals, whereas the values around zero characterize higher regularity [17,24].

3.3.4. Detrended Fluctuation Analysis of EDA

Detrended fluctuation analysis (DFA) is a scaling analysis method for evaluating the statistical self-similarity properties of the time series. DFA is used to confirm the existence of persistent long-term range associations in EDA data. Exponent α represents the slope of the trend line relating log $\underline{F(n)}$ to log (n) in the range of time-scales. It is estimated by linear regression. Generally, two scaling exponents—α1 and α2 are evaluated, which represent relationships over short (≤30 s) and long (>30 s) timescales, respectively [35]. At first, the analyzed data (length of N) was integrated and divided into boxes of equal length n. In individual boxes of n, a least square line fits the signals. The y coordinate of the straight stretches is marked by $y_n(k)$. Subsequently, we subtracted the local trend $y_n(k)$ from y(k) in each box, i.e., we detrended them. Finally, the root-mean-square fluctuation of this combined and detrended signals was computed according to Lanata et al. (2012) [16]:

$$F\ (n) = \sqrt{\frac{1}{N}\ \sum_{k=1}^{N}[y(k) - y_n(k)]^2}, \tag{5}$$

Figure 1 summarizes the EDA evaluated parameters using linear and nonlinear analysis.

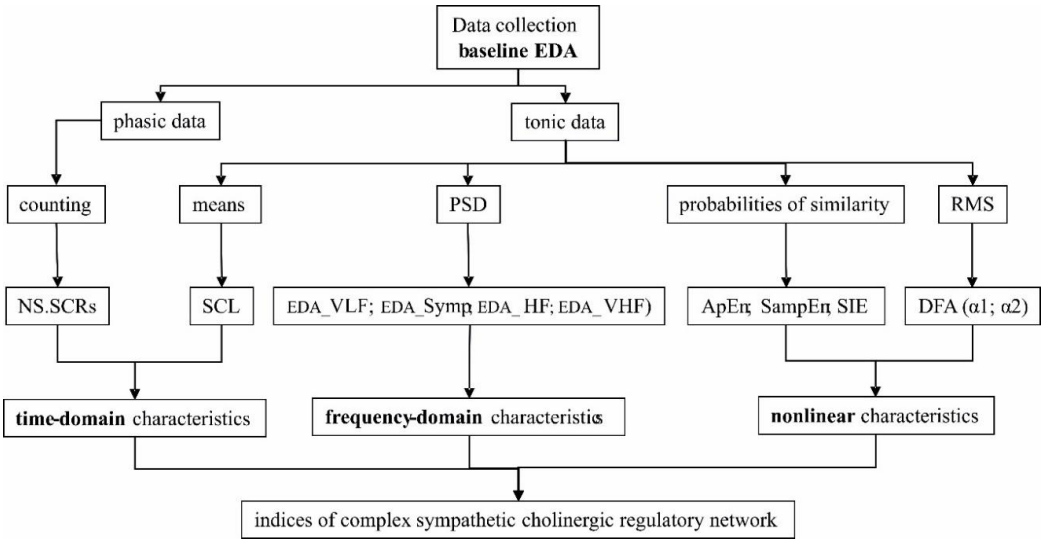

Figure 1. Evaluation of electrodermal activity (EDA) parameters. SCL—skin conductance level; NS.SCRs—nonspecific skin conductance responses; PSD—power spectral density; EDA-VLF—electrodermal activity in very low frequency band; EDA-Symp—EDA in the sum of low and high 1 frequency bands; EDA-HF—EDA in high 2 frequency band; EDA-VHF—EDA in very high frequency band; ApEn—approximate entropy; SampEn—sample entropy; SIE—symbolic information entropy; RMS—root mean square; DFA—detrended fluctuation analysis; α1, α2—indices of detrended fluctuation analysis short-term and long-term correlations, respectively.

4. Statistical Analysis

Statistical analysis was performed by SYSTAT (SSI, Richmond, CA, USA). The Lilliefors´ normality statistical test was used for evaluation data distributions (Gaussian/non-Gaussian). The Mann–Whitney test was used for between-group comparison of EDA indices and the peripheral temperature (PT) because data were not normally distributed. Anthropometric parameters were normally distributed and analyzed by unpaired Student´s *t*-test. The associations between the parameters of EDA and the peripheral temperature, and correlations between anthropometric characteristics (BMI, BSA) and EDA parameters were analyzed using Spearman´s rank-order correlation test. A value of $p < 0.05$ (two-tailed) was considered statistically significant. Anthropometric parameters were expressed as a mean ± standard error of the mean (SEM). Values of EDA and PT were expressed as median (interquartile range).

5. Results

Anthropometric characteristics of both groups (AN and controls) are summarized in Table 1.

Table 1. Anthropometric characteristics of groups.

	Anorexia Nervosa (N = 25)	Controls (N = 25)	*p* Value
Age (years)	14.76 ± 0.38	15.12 ± 0.32	0.580
Weight (kg)	46.04 ± 1.56	57.76 ± 2.58	0.0001
Height (m)	1.65 ± 1.28	1.66 ± 1.90	0.816
BMI (kg/m^2)	16.88 ± 0.48	20.88 ± 0.66	0.0001
BSA (m^2)	1.45 ± 0.03	1.62 ± 0.04	0.001
WC (cm)	64.79 ± 1.41	75.80 ± 1.99	0.0001

Values are expressed as mean ± SEM. BMI—body mass index; BSA—body surface area; WC—waist circumference. *p* values express the comparison between groups. Probabilities $p < 0.05$ were considered as significant.

5.1. Comparison between Groups of Time-Domain EDA Parameters

Index SCL was significantly lower in the AN group compared to controls ($p = 0.041$). No significant differences were found in index NS.SCRs (Table 2).

Table 2. Parameters of electrodermal activity and peripheral temperature.

Parameter	Anorexia Nervosa (n = 25)	Controls (n = 25)	*p* Value
Time-domain indices of EDA			
SCL (μS)	0.67 (0.32–2.21)	2.10 (0.97–3.23)	0.041
NS.SCRs	0.50 (0.00–1.00)	1.00 (0.00–2.00)	0.257
Frequency-domain indices of EDA			
EDA-VLF (μS^2)	18.85 (0.28–56.66)	238.84 (41.39–798.81)	0.001
EDA-Symp (μS^2)	16.33 (3.09–103.79)	815.60 (125.69–1427.03)	0.0001
EDA-HF2 (μS^2)	1.45 (0.37–5.94)	1.73 (0.44–5.07)	0.684
EDA-VHF (μS^2)	1.45 (0.37–5.94)	0.21 (0.03–1.07)	0.765
Nonlinear indices of EDA			
ApEn	0.59 (0.41–0.82)	0.67 (0.45–0.82)	0.674
SampEn	0.48 (0.28–1.18)	0.48 (0.27–0.81)	0.562
SIE	2.14 (2.06–2.65)	2.35 (1.51–2.76)	0.734
$\alpha 1$	1.01 (0.65–1.20)	0.94 (0.77–1.17)	0.779
$\alpha 2$	0.49 (0.07–0.73)	0.11 (0.05–0.31)	0.032
Index of peripheral temperature			
PT (°C)	30.53 (25.93–33.46)	31.43 (28.94–32.54)	0.487

5.2. Comparison between Groups of Spectral-Domain EDA Parameters

Index EDA in the very low frequency band was significantly lower for AN group compared to HC ($p = 0.001$). EDA-Symp was significantly lower during baseline for AN vs. HC groups ($p = 0.0001$). No significant differences were found in indices EDA in the high and very high frequency bands (Table 2).

5.3. Comparison between Groups of Nonlinear EDA Parameters

Only nonlinear index $\alpha 2$ was significantly higher for AN group compared to HC ($p = 0.032$). No significant differences were found in remaining nonlinear parameters—ApEn, SampEn, SIE and $\alpha 1$ between groups (Table 2).

5.4. Comparison between Groups of Peripheral Temperature

Peripheral temperature was without significant differences between groups (Table 2).

Values are expressed as median (interquartile range). SCL—skin conductance level; NS.SCRs—nonspecific skin conductance responses; EDA-VLF—electrodermal activity in very low frequency band; EDA-Symp—EDA in the sum of low and high 1 frequency bands; EDA-HF—EDA in high 2 frequency band; EDA-VHF—EDA in very high frequency band; ApEn—approximate entropy; SampEn—sample entropy; SIE—symbolic information entropy; $\alpha 1$, $\alpha 2$—indices of detrended fluctuation analysis short-term and long-term correlations, respectively; PT—peripheral temperature. The *p* value expresses the comparison between groups. Probabilities $p < 0.05$ were considered to be significant.

5.5. Correlation Analysis

Correlation analysis in the total group revealed significant positive correlations between BMI and SCL, and between BMI and PT ($r = 0.292$, $p = 0.042$; $r = 0.302$, $p = 0.041$, respectively). Index SCL was significantly positively correlated with indices of spectral-domain EDA-VLF and EDA-Symp ($r = 0.556$, $p = 0.0001$; $r = 0.277$, $p = 0.050$, respectively). No significant correlations were found in the remaining parameters.

6. Discussion

To the best of our knowledge, this study was the first to describe features of the sympathetic cholinergic regulatory system using spectral and nonlinear characteristics of EDA in AN adolescents before starting a treatment. The results can be summarized as follows: (1) several indices of time (SCL)

and frequency (EDA-VLF and EDA-Symp) analysis were significantly lower, indicating insufficient sympathetic cholinergic activity already in adolescent anorexia nervosa; (2) in contrast, the index of DFA–α2 showed a different pattern compared to the time and spectral EDA characteristics—it was increased in anorectic patients thus providing independent and novel information related to altered complexity in sympathetic cholinergic regulatory network in anorexia; (3) correlation analysis revealed that BMI is positively associated with SCL and PT indicating possible alteration of sympathetically mediated and thermoregulatory mechanisms in AN, which could be induced by extensive reduction in body weight. Several mechanisms are proposed.

First, reduced SCL is in accordance with other studies that revealed a lower mean of tonic EDA in AN adults [18,20] and extend these findings into the adolescent age group. Additionally, our study revealed statistically important decrease of EDA in spectral-domain (EDA-VLF, EDA-LF and EDA-Symp) in AN compared to controls. Moreover, we found a positive correlation between SCL and EDA-VLF, and between SCL and EDA-Symp. Thus, we assume that EDA evaluation by SCL, EDA-VLF and EDA-Symp indices represents a sufficient non-invasive tool for assessment of sympathetic cholinergic underactivity linked to AN. Our previous study revealed reduced sympathetic activity based on blood pressure variability analysis in newly diagnosed AN girls, indicating nutritional trajectory as an adaptation process associated with bodyweight control from "normal weight to underweight" [8]. Thus, we assume that acute phase of adolescent AN is characterized by sympathetically mediated autonomic hypoactivation which could only partially be ascribed to the reduced body weight measured by BMI associated with reduced basal metabolism. Specifically, neurobiological features such as hormonal deficiencies (e.g., decreased level of leptin and increased level of ghrelin), neurotransmitters (e.g., serotonin and dopamine) [36–38] and electrolyte dysfunctions (e.g., mainly potassium) [39] could play an important role in AN-linked sympathetic underactivity. Notably, leptin as an important molecular signal of energy abundance is closely linked to sympathetic regulation [40,41], and reduced leptin concentrations associated with sympathetic hypoactivity were found in anorexia nervosa [42].

Further, psychopathological features linked to AN could also importantly contribute to sympathetic hypoactivity in AN. A recent study found an inverse correlation between the negative beliefs concerning the consequences of not controlling thoughts and SCL, suggesting that metacognitive variables (i.e., the inescapability of negative thoughts and the need for thought control) could have a key role in modulating patients' sympathetic hypoactivation. Moreover, this worry could be considered a cognitive strategy to avoid intense emotions. From this perspective the dysfunctional metacognitions about worry might yield also a reduced sympathetic activity in anorexia nervosa [20]. Therefore, it is questionable whether AN-linked sympathetic hypoactivity is predominantly a result of neurobiological changes or is more a reflection of psychopathological features associated with AN.

Due to the fact that biological signals are very difficult to interpret and with various timing and diverse structure, we used nonlinear methods of analysis, which better characterize complex qualitative features of the system. Contrary to time and frequency domain indices, we revealed that the index α2 was significantly higher in AN group compared to controls. Thus, we assume that the α2 index could provide independent information about the complexity of the sympathetic cholinergic regulatory network compared to other time and frequency parameters of EDA. With respect to another biosignal (heart rate), Ishizawa et al. (2008) revealed increased complexity of heart rate variability based on DFA in AN group [9]. Additionally, Jelinek et al. (2017) found an increase of α2-HRV during orthostasis in AN compared to controls [43]. It seems that this important issue remains for further research to elucidate pathways linking sympathetic complexity and anorexia nervosa.

In light of these findings, we could speculate about the complex mechanisms. The excitatory and inhibitory influences of the sympathetic regulation derive from different structures of the central nervous system. We assume that the control mechanisms and neural pathways' influence on the central regulation of EDA are complex. Boucsein et al. (1992) define two potential independent paths affecting sweat production and thus skin conductance: A) cortical level—central control of EDA applies to inclination of cortical centers and basal ganglia as brain structures encompassed in

motor control [44]. Notably, cortical pathways encompass excitatory control via pyramidal pathways and the premotor cortex [26,44,45]; B) subcortical level—EDA control encompasses effects from the hypothalamus and the limbic system related to thermoregulatory sweating. Additionally, the inhibitory effects of EDA are mediated by the hippocampus, whereas the excitatory effects are mediated by the amygdala [26,44,45]. Furthermore, the EDA is changed by control effects of reticular formation in the brainstem [26]. Therefore, we assume that the complexity of the brain regulatory regions (prefrontal cortex, hypothalamus) and the existence of feedback loops create a chaotic system influencing EDA.

Conversely, decreased prefrontal cortex (PFC) activity has often been observed among AN patients compared to healthy controls using functional neuroimaging techniques [46–49]. The PFC is a key area for executive functioning, which includes inhibitory control, working memory, and planning. Behavioural studies have revealed that AN patients often have complications with tasks requiring inhibitory control, including response inhibition and task switching [50]. Thus, inhibitory control might be a key element influencing the salient characteristics of AN. Functional imbalance between reward and inhibitory control in the cerebral cortex, especially the PFC, might be related to AN susceptibility and pathology. However, regional structural relevance regarding functional abnormalities in AN remains largely unknown [51–53]. Moreover, the PFC is one of the last brain regions to fully develop relative to subcortical areas [53]. PFC organization undergoes dynamic variations in terms of synaptic density throughout prepubescence and adolescence [54]. This might represent one of key pathomechanisms leading to both AN onset in adolescence and abnormalities in complex feedback regulatory loops resulting in AN-linked sympathetic disruption.

Taken together, the conventional and nonlinear EDA parameters reveal different features of the complex sympathetic cholinergic system consisting of multiple feedback regulatory loops in adolescent AN. While time and spectral parameters of EDA characterize quantitative features of sympathetic cholinergic regulation indicating sympathetic hypoarousal in adolescent AN, the parameter of DFA–α2 could provide novel and distinct information about the qualitative feature complexity of the sympathetic regulatory network, as well.

7. Conclusions

Our study revealed decreased time and spectral indices of EDA indicating sympathetic underactivity in never-treated AN presenting at adolescent age. However, the nonlinear parameter α2 showed a different pattern, indicating abnormal complexity of the sympathetic cholinergic regulatory network in adolescent AN. We suggest that the complex EDA analysis seems to be a sensitive tool for evaluating the activity of the sympathetic cholinergic modulation in AN adolescents. Further, the discrete alteration of the complex sympathetic regulatory network indexed by EDA might represent a risk factor for greater cardiovascular complications already present in adolescent AN.

Future studies should be directed to clarify pathomechanisms linking AN and increased cardiovascular risk, and reveal a complex profile of the sympathetic nervous activity in anorexic patients, particularly in adolescent age. Further clinical research is necessary to identify biomarkers for the assessment of sympathetic complex dysfunction, which could be used for early prevention and personalized treatment of cardiovascular diseases in AN patients.

8. Limitations of the Study

In present study, the cohort consisted of a relatively small homogenous sample of female adolescents with newly diagnosed AN; therefore, it is needs to be validated in a larger cohort with respect to gender. Further, EDA represents a noninvasive index of the sympathetic cholinergic system; therefore, it is not possible to apply these results in terms of general sympathetic dysregulation associated with anorexia nervosa. In this aspect, future research based on continual analysis of additional parameters evaluating the sympathetic control and exploring complex relations between EDA signal and other sympathetically-mediated biosignals (e.g., pre-ejection period, blood pressure variability) under physiological and pathological conditions is needed.

Author Contributions: Z.V., researcher in the field of normal and pathological physiology. She wrote and contributed to the preparation of all parts of the original and revised manuscript and participated in final version of the manuscript. L.B.O., N.S., young researchers in the field of normal and pathological physiology. A significant contribution of all named authors was searching and selection of the scientific studies according to the topic of manuscript, writing and final editing of the original and revised manuscript. I.O., I.H., D.C., clinicians in pediatric psychiatry. They significantly participated in writing and final supervision of the manuscript parts related to the pedopsychiatric topic. S.K., researcher in the field of midwifery and I.F. researcher in the field of nursing. They were involved in searching and selection of the scientific studies according to the topic of manuscript. I.T., professor of normal and pathological physiology, and head of research team. She contributed significantly to the preparation of all parts of the manuscript, final version, supervision and approval of the original and revised manuscript. All authors have read and agreed to the published version of the manuscript.

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Abbreviations

AN	anorexia nervosa
EDA	electrodermal activity
DFA	detrended fluctuation analysis
ANS	autonomic nervous system
IAPS	International Affective Picture System
SCR	skin conductance response
HC	healthy controls
BMI	body mass index
DSM-5	Diagnostic and Statistical Manual of Mental Disorders
Ag-AgCl	silver-silver chloride
WC	waist circumference
BSA	body surface area
h	height
w	weight
SCL	skin conductance level
NS.SCRs	non-specific skin conductance responses
PSD	power spectral density
EDA-VLF	electrodermal activity in very low frequency band
EDA-Symp	electrodermal activity in the sum of low and high 1 frequency bands
EDA-HF	electrodermal activity in high 2 frequency band
EDA-VHF	electrodermal activity in very high frequency band
ApEn	approximate entropy
SampEn	sample entropy
SIE	symbolic information entropy
RMS	root mean square
$\alpha 1$	indices of detrended fluctuation analysis short-term correlation
$\alpha 2$	indices of detrended fluctuation analysis long-term correlation
VLF	very low frequency band
HF	high frequency band
VHF	very high frequency band
N	number of points
r	tolerance range
m	length of sequence
L	length of vector
p	probability
y	coordinate of the strait stretches
F	fluctuation of combined and detrended signal
SEM	standard mean error
PT	peripheral temperature
PFC	prefrontal cortex

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