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RESEARCH ARTICLE

Autonomic nervous system function in women with anorexia nervosa

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Abstract

Purpose Abnormalities in autonomic function have been observed in people with anorexia nervosa. However, the majority of investigations have utilised heart rate variability as the sole assessment of autonomic activity. The current study utilised a variety of methodologies to assess autonomic nervous system function in women with a current diagnosis of anorexia, a past diagnosis of anorexia who were weight-restored, and healthy controls.

Methods The sample included 37 participants: 10 participants with anorexia, 17 weight-restored participants (minimum body mass index > 18.5 for minimum of 12 months) and 10 controls. Assessments of autonomic function included muscle sympathetic nerve activity (MSNA) using microneurography, heart rate variability, baroreflex sensitivity, blood pressure variability, head-up tilt table test, sudomotor function and assessment of plasma catecholamines.

Results MSNA (bursts/min) was significantly decreased in both anorexia (10.22 ± 6.24) and weight-restored (17.58 ± 1.68) groups, as compared to controls (23.62 ± 1.01, $p < 0.001$ and $p = 0.033$, respectively). Participants with anorexia had a significantly lower standard deviation in heart rate, lower blood pressure variability and decreased sudomotor function as compared to controls. Weight-restored participants demonstrated decreased baroreflex sensitivity in response to head-up tilt as compared to controls.

Conclusion Women with a current or previous diagnosis of anorexia have significantly decreased sympathetic activity, which may reflect a physiological response to decreased energy intake. During the state of starvation, women with anorexia also displayed decreased sudomotor function. The consequences of a sustained decrease in MSNA are unknown, and future studies should investigate autonomic function in long-term weight-restored participants to determine whether activity returns to normal.

Keywords Anorexia nervosa · Muscle sympathetic nerve activity · Microneurography · Autonomic nervous system · Orthostatic intolerance · Sudomotor function

Introduction

Anorexia nervosa (AN) is a disorder characterised by an intense fear of gaining weight and a distorted self-perception of body image, culminating in restriction of food intake and an extremely low body weight [1]. With a typical onset in adolescence [2], AN has an estimated lifetime prevalence of 1.7% in the general population [3]. The most profound risk factor for AN is female sex, with epidemiological studies reporting nine cases in females for every one case in

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males [4–6], and the mortality rate associated with AN, approximately six times that of the general population [7, 8], is amongst the highest of any psychiatric disorder [9]. A significant contributor to the morbidity and mortality rate is cardiovascular complications encompassing structural, conduction and haemodynamic abnormalities [10–12]. Moreover, cardiovascular risks are not confined to the state of starvation alone; arrhythmias and congestive heart failure can occur during the process of re-feeding [13, 14]. While the mechanism of increased cardiac risk attributable to AN remains to be fully understood, there is growing interest in the role of autonomic regulation in contributing to cardiovascular events.

A recent systematic review summarised previous investigations into autonomic nervous system (ANS) activity in patients with acute AN and in those with a previous diagnosis who were weight-restored, which suggested that abnormalities in ANS function were present in acute AN and tended to normalise following weight restoration [15]. The majority of assessments of ANS activity in AN to date have utilised heart rate variability (HRV) as an index of cardiac vagal regulation, with some indicating parasympathetic dominance and increased beat-to-beat variance in heart rate (HR) [16]; yet there have been conflicting findings [17]. While HRV provides some insight into vagal activity, it provides limited information about sympathetic activity [18, 19]. Low-frequency HR spectral power is often interpreted as a marker of sympathetic activity, yet is unrelated to direct assessments of sympathetic activity such as muscle sympathetic nerve activity (MSNA) and cardiac noradrenaline spillover to plasma [20]. Moreover, an experimental model of heart failure demonstrated no relation between measures of HRV and directly recorded cardiac nerve activity [21]. Other assessments of ANS activity in people with AN have included blood pressure variability (BPV), baroreflex sensitivity (BRS), catecholamine assessment, haemodynamic response to an orthostatic challenge, and skin conductance. Assessment of BPV and BRS at rest illustrated increased parasympathetic control over the heart in AN as compared to controls [22–24]. Similarly, previous observations revealed a trend towards decreased circulating plasma noradrenaline levels in acute AN, which normalised following weight restoration [15].

The orthostatic stress test provides a window into autonomic regulation through the baroreceptor reflex control of BP and HR [25–27]. Conditions related to orthostatic intolerance such as orthostatic hypotension, syncope and postural tachycardia syndrome (POTS) represent an acute autonomic and haemodynamic perturbation and have also been reported in AN [10]. Observations of HRV, BPV, BRS and plasma noradrenaline responses to a head-up tilt (HUT) have provided some evidence of possible abnormal cardiac autonomic regulation in individuals with AN [28–30], bearing in mind that plasma noradrenaline levels in response to a HUT may not be a reliable diagnostic test for autonomic failure [31]. Skin conductance has not been commonly utilised as an assessment of ANS activity in AN, but some studies have demonstrated, albeit not conclusively, decreased skin conductance levels (SCL) in people with AN [32, 33].

In sum, while various investigations of ANS function in AN have been conducted, few have employed multiple methodologies simultaneously, in contrast to recommendations that multiple assessments should be undertaken in order to assess ANS activity comprehensively [25]. Furthermore, to our knowledge, there has been no direct assessment of sympathetic activity using microneurographic measurement of MSNA in AN. MSNA provides a direct measure of sympathetic outflow [25] and has been shown to be closely linked with body weight [34], thermoregulation [35], and cardiac [36] and metabolic function [37]. Another deficit in the field has been a general lack of weight-restored AN comparison groups, as well as comparison with non-AN controls. The current study aimed to address these limitations by assessing ANS function using a comprehensive battery of assessments, including direct measurement of MSNA, in both individuals in the acute stage of AN and those who were weight-restored. Given the increased prevalence of AN in women, as well as sex differences in body composition in general [38, 39] and in AN [40, 41], the current study aimed to assess ANS function in women with AN. It was hypothesised that women with a current diagnosis of AN would display increased parasympathetic activity and decreased sympathetic activity through increased HRV, decreased BPV, increased BRS and decreased MSNA. It was anticipated that ANS activity in participants with a previous diagnosis of AN who were weight-restored would not differ from that of controls.

Methods

Participants

Three groups of participants were included: 10 with a current diagnosis of AN, 17 with a previous diagnosis of AN who were weight-restored (AN-WR) and 10 healthy controls (HCs). HCs were recruited through public advertisements, whereas AN and AN-WR participants were recruited through public advertisements and the Body Image Registry at Swinburne University of Technology in Melbourne, Australia.

All participants were required to fulfil the following inclusion criteria: female, over the age of 18 years and English-speaking. Exclusion criteria included pregnancy, current drug or alcohol use disorder, neurological illness, and history of traumatic brain injury or a psychotic condition. AN

participants were required to have a current diagnosis of AN according to DSM-5 [Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition] criteria [1]; AN-WR participants were required to have a previous but not current diagnosis of AN, with a BMI > 18.5 for at least 12 months. Current and previous diagnoses of AN were confirmed through a comprehensive assessment using the Mini-International Neuropsychiatric Interview (M.I.N.I.) 7.0.2 [42] by a trained investigator under the guidance of a consultant psychiatrist in accordance with DSM-5 criteria. HCs had no known chronic disease (including cardiovascular disease) and no personal history or first-degree relative with a major psychiatric illness, including eating disorders, which was confirmed with the M.I.N.I.

The research protocol complied with the Declaration of Helsinki and was approved by the human research ethics committees at Swinburne University of Technology, The Melbourne Clinic and St Vincent's Hospital; all in Melbourne, Australia. Written informed consent was obtained from each participant prior to the study.

Procedures

All participants attended a single assessment session at 9:00 am after having fasted for at least 10 h and abstained from caffeine for 18 h, alcohol for 24 h and exercise for 36 h prior to participation. Demographic details, eating disorder status, and treatment history and detailed medical history were collected.

Biochemistry

Fasting blood samples were drawn from a cannula placed in an antecubital vein for biochemical analysis of catecholamines, which was collected in an EDTA tube and centrifuged at 3500 rpm for 15 min at 4 °C. Plasma was stored at −80 °C for subsequent analysis. Following blood collection, participants were offered a light, low-glucose breakfast of approximately 1000 kJ approximately 90 min prior to MSNA recording.

Anthropometric measurements

Height and weight were measured in indoor clothes without shoes. Height was measured using a stadiometer. Weight was assessed using the Tanita scale [43] that uses bioelectrical impedance technology to calculate body mass index (BMI).

Brachial blood pressure and heart rate

Brachial BP and HR were measured after 10-min rest using a calibrated automated sphygmomanometer (Omron model HEM-7121). Three consecutive measurements were taken with patients in an upright seated position, and the mean value was recorded as the patient's systolic (SBP) and diastolic blood pressure (DBP) and HR.

Assessments of autonomic nervous system

Assessments of ANS function were conducted on the same morning in a temperature-controlled (22 °C) research room. Participants lay in a semi-supine position for BP, HR and MSNA recordings. After a resting period of at least 10–15 min, all parameters were continuously recorded over a 20-min period while participants were asked to breathe at their spontaneous frequency.

(i) Muscle sympathetic nerve activity

Multi-unit postganglionic sympathetic activity was recorded using microneurography, with participants resting in a semi-supine position as described previously [44]. A tungsten microelectrode (FHC, Bowdoin, ME, USA) was inserted directly into the right peroneal nerve just below the fibular head, and a subcutaneous reference electrode was positioned 2–3 cm from the recording site. The needle was adjusted until satisfactory spontaneous MSNA was observed in accordance with previously described criteria [45]. The nerve signal was amplified (350,000), filtered (bandpass 700–2000 Hz) and integrated. After an acceptable nerve recording site was obtained, resting measurements were recorded over a 20-min period and averaged. MSNA recordings have been shown to be stable over 3 months [46].

(ii) Blood pressure and heart rate

During MSNA recording, beat-to-beat BP was continuously measured using a non-invasive BP device (NIBP; ADInstruments, Bella Vista, Australia) and HR was determined using a three-lead echocardiogram (ECG). MSNA, BP and ECG were digitised with a sampling frequency of 1000 Hz (PowerLab recording system, model ML 785/8SP; ADInstruments).

(iii) Head-up tilt table test

Following the MSNA recording, participants underwent a passive tilt table test following the protocol recommended by the American Heart Association [47]. Participants lay in a supine position on a table with footboard support and were gently secured to the table for safety purposes. Following a resting period of 10 min, the table was tilted upright to 80° within 15 s. The duration of the tilt was 10 min. Beat-to-beat arterial BP measurements and ECG were continuously measured. BP values recorded with the finger sphygmomanometer were compared with values obtained every 2 min from a standard cuff sphygmomanometer to confirm

accuracy. Following the tilt test, participants remained supine for a recovery period of 6 min.

(iv) Sudomotor function assessment

An evaluation of sweat gland function was conducted through reverse iontophoresis and chronoamperometry using the Sudoscan device (Impeto Medical, Paris, France). Participants placed the palms of their hands and the soles of their feet on stainless steel electrodes, and an incremental low direct voltage (< 4 V) was applied for 2 min. Electrochemical skin conductance (ESC), a measure of sudomotor function, was obtained from the ratio between the current that was measured and voltage applied. Quantitative results were expressed as ESC (microsiemens, μS) for the hands and feet, and the hand and feet mean asymmetry measure, given as a percentage (%), was calculated by the difference between right and left ESC divided by the higher of the two ESCs. Sudomotor dysfunction is evaluated according to the ESC measured on the feet: > 60 μS = no dysfunction; 60–40 μS = moderate dysfunction; and < 40 μS = severe dysfunction [48]. A cardiac autonomic neuropathy (CAN) risk score (CAN-RS) derived from the ESC values and demographic data (BMI and age) was calculated using an algorithm described previously [49].

Analyses

i. MSNA analysis

Sympathetic bursts were counted manually by visual inspection of the signal, and the number of bursts was averaged over the 20-min period. Bursts were identified by taking into account the timing of the burst occurrence (maximum 1 burst per heart cycle, occurring towards the end of corresponding diastole) as well as a 3:1 signal-to-noise ratio [50]. The MSNA was expressed as burst frequency (burst/min) and burst incidence (bursts/100 heartbeats).

ii. Heart rate variability

HRV was assessed from the resting ECG recording and was determined using commercially available software (HRV Module for Chart 5 Pro; ADInstruments, Bella Vista, Australia). Parameters derived were standard deviation of normal to normal intervals (SDNN) and standard deviation of HR (SD rate) in the time domain analysis. Low frequency (LF: 0.04–0.15 Hz) and high frequency (HF: 0.15–0.4 Hz) in the frequency domain analysis were expressed as percentage and normalised units. Additionally, the LF/HF ratio was calculated in the HRV analysis. Data are reported as total power and % of total power. HRV was calculated over a minimum 5-min period of rest.

iii. Cardiac baroreflex sensitivity

BRS was assessed using the sequence method [51]. This procedure identifies the 'spontaneous' sequences of three or more consecutive beats in which systolic BP progressively rose and cardiac interval progressively lengthened (type 1 sequences), or systolic BP progressively fell and cardiac interval progressively shortened (type 2 sequences), with a lag of one beat. For each sequence, the linear correlation coefficient between cardiac interval and systolic BP was computed and the sequence validated when $r > 0.85$. The slope between cardiac interval and systolic BP was calculated for each validated sequence and expressed as milliseconds/mmHg. The baroreflex efficacy index (BEI) [52] was assessed as the total number of cardiac intervals/systolic BP sequences divided by the total number of systolic BP ramps. Recordings were averaged over a minimum 5-min period of rest, tilt and recovery conditions.

iv. Blood pressure variability

The variability in BP was assessed using the standard deviation (SD) of the beat-to-beat BP recording and calculated using every cardiac cycle [53, 54]. Recordings were averaged over a minimum 5-min period of rest, tilt and recovery conditions.

v. Catecholamine analysis

Plasma concentrations of adrenaline, noradrenaline and its intraneuronal metabolite 3,4-dihydroxyphenylglycol (DHPG) were determined by high-performance liquid chromatography with coulometric detection [55] following extraction from plasma samples using alumina adsorption, and the concentrations were corrected for loss during extraction using recoveries of an internal standard.

Data analyses

Statistical analyses were performed using SPSS (IBM, SPSS Statistics version 26) and data are presented as the mean ± SD. For normally distributed data, between-group comparisons of continuous variables were performed using analyses of variance (ANOVAs) and Welch ANOVA when there was not homogeneity of variances. Tukey and Games–Howell post hoc tests were used respectively for multiple comparisons. Non parametric Kruskal–Wallis one-way ANOVA on ranks was used to conduct between-group comparisons on non-normally distributed data, with Dunn's procedure [56] used for pairwise comparisons. Upon identification of outliers, the analyses were run with and without the outlier. Outliers were not removed from the analyses, as they did not impact the results. Pearson's correlation analyses were performed to assess the relationship between variables of interest. When data were

non-normally distributed, non-parametric Spearman's correlation was used. For all analyses, significance was set at $p < 0.05$.

Results

Participant demographic and clinical characteristics are presented in Table 1. Groups did not differ in age. Participants with a current diagnosis of AN had a significantly longer illness duration than those who were WR, and there were significantly more participants taking psychotropic medication in the AN and AN-WR groups than in the HC group. The AN group had significantly lower HR and SBP than HCs (see Table 1).

Muscle sympathetic nerve activity

MSNA was successfully recorded in 32 participants (10 AN, 12 AN-WR, 10 HCs). Examples of MSNA recordings from each group are shown in Fig. 1.

Participants with AN demonstrated significantly fewer bursts/min (10.22 ± 6.24 vs 23.62 ± 1.01, respectively, $p < 0.001$; see Fig. 2a) and bursts/100 heartbeats (18.59 ± 3.37 vs 37.14 ± 2.67, respectively, $p = 0.001$; see Fig. 2b) than HCs. Participants who were WR demonstrated significantly fewer bursts/min than HCs (17.58 ± 1.68 vs 23.62 ± 1.01, respectively, $p = 0.033$; see Fig. 2a). There was a trend for a gradient of bursts/100 heartbeats among the three groups; WR participants trended towards fewer bursts/100 heartbeats than HCs (27.60 ± 2.87 vs 37.14 ± 2.67, respectively, $p = 0.074$) and more bursts/100 heartbeats than AN (27.60 ± 2.87 vs 18.59 ± 3.37, respectively, $p = 0.095$): see Fig. 2b.

Table 1 Comparison of demographic and clinical characteristics across groups

	AN M ± SD or *N* (%)	AN-WR M ± SD or *N* (%)	HC M ± SD or *N* (%)	*p* value
Sex	10F	17F	10F	
Age (years)	31.64 ± 11.25	25.07 ± 4.87	27.44 ± 6.07	0.097
BMI	15.57 ± 1.69	21.64 ± 2.14 [a]	21.40 ± 1.84 [a]	**< 0.0005**
Age of AN onset (years)	16.40 ± 3.53	15.82 ± 2.16	–	0.601
Duration of AN (years)	14.58 ± 10.20	3.74 ± 3.10	–	< **0.001**
Duration of WR (years)		3.82 ± 2.77		
Current/past smoker (*n*)	–	4 (23.5%)	2 (20.0%)	0.607
Psychotropic medication treatment (*n*)	6 (60.0%)	9 (52.9%)[c]	–[b]	**0.006**
HR (bpm)	57.7 ± 11.6	68.8 ± 10.3[b]	69.0 ± 8.01[b]	**0.019**
SBP (mmHg)	98.2 ± 11.7	108.3 ± 6.3[b]	112.8 ± 9.4[a]	**0.002**
DBP (mmHg)	66.3 ± 8.19	70.0 ± 6.28	72.7 ± 7.22	0.143

Bold indicates a significant difference between the three groups

AN anorexia nervosa; *AN-WR* weight-restored; *HC* healthy controls; *BMI* body mass index; *M* mean; *SD* standard deviation; *HR* heart rate; *SBP* systolic blood pressure; *DBP* diastolic blood pressure

[a] $p < 0.001$; compared to AN

[b] $p < 0.05$; compared to AN

[c] $p < 0.05$, compared to HC

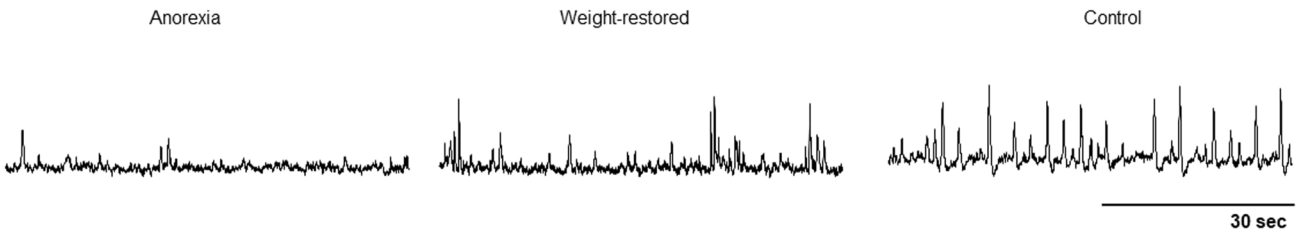

Fig. 1 Original recordings of MSNA from a representative participant in each group

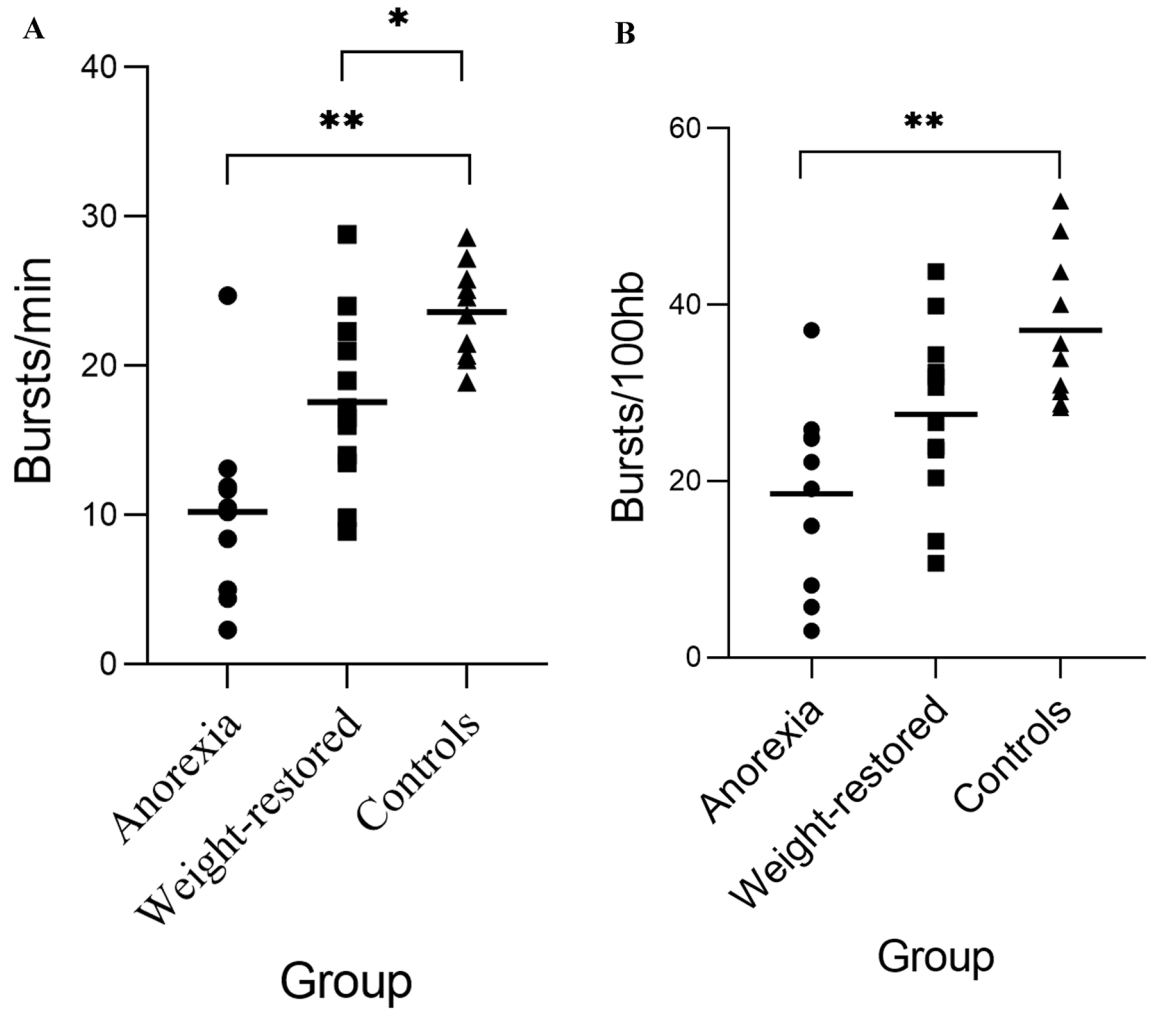

Fig. 2 **a** MSNA bursts/min across groups; **b** MSNA bursts/100 heartbeats across groups. * Denotes significant difference between groups, $p < 0.05$; ** denotes significant difference between groups, $p < 0.005$. *hb* heartbeat

Univariate correlation analyses between MSNA bursts/min and clinical and anthropometric variables revealed two significant associations. MSNA burst frequency was significantly associated with BMI ($r = 0.485$; $p = 0.005$; see Fig. 3) and DBP ($r = 0.361$; $p = 0.042$). There were no associations between MSNA and duration of illness or other anthropometric variables. Supplementary Fig 1. groups the participants further according to psychotropic medication treatment status.

Blood pressure and heart rate

Heart rate variability

Participants with AN had significantly lower standard deviation in the HR than both AN-WR ($p = 0.019$) and HCs ($p = 0.004$) (see Table 2). There was no difference between groups in other measures of HRV, including those assessed in the frequency domain.

Blood pressure variability

BPV was assessed during rest, tilt and recovery conditions. Participants with AN demonstrated significantly lower variability in SBP ($p = 0.013$) and DBP ($p = 0.002$) than HCs during resting conditions (see Table 2). Moreover, the AN group had significantly lower variability in DBP ($p = 0.004$) than HCs during recovery from HUT.

Participants who were WR displayed significantly lower variability in DBP than HCs during resting and recovery conditions (both $p = 0.037$).

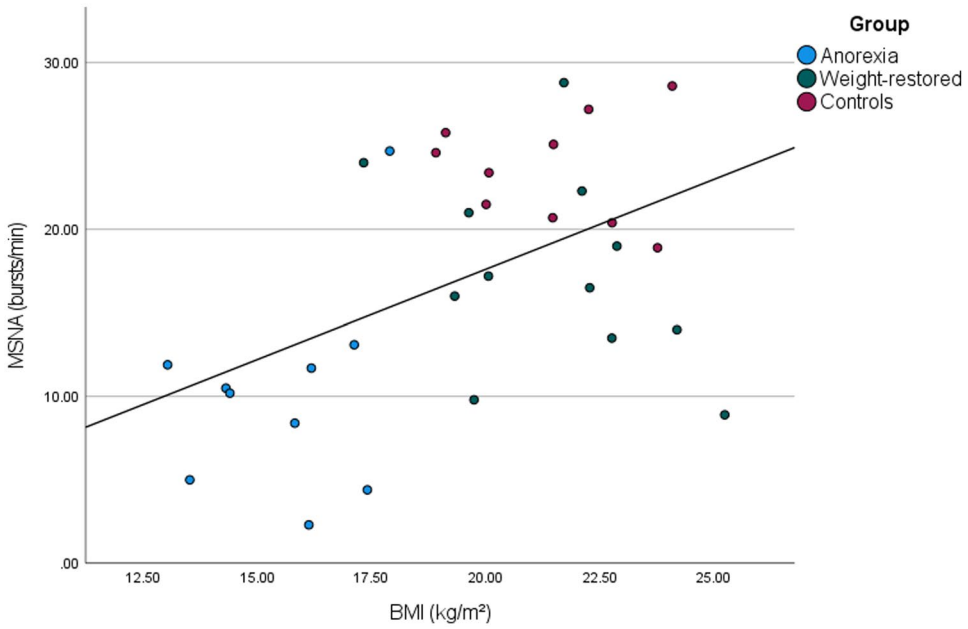

Fig. 3 Correlation between MSNA bursts/min and BMI across entire sample ($r = 0.485$; $p = 0.005$). *BMI* body mass index; *MSNA* muscle sympathetic nerve activity

Cardiac baroreflex sensitivity

BRS was assessed during rest, tilt and recovery conditions. There was no difference in BRS between groups at rest or during recovery from HUT (see Table 2). However, the AN-WR group demonstrated significantly lower mean slope during the tilt, than HCs ($p = 0.012$).

Head-up tilt table test

Three AN-WR participants experienced syncope or became unwell during the HUT and were immediately returned to a supine position. There were no significant differences between the average changes in HR, SBP or DBP between groups (see Table 3). However, the AN-WR reached their maximum HR significantly faster than the HCs during the 10-min HUT ($p = 0.005$).

Within the AN-WR group, univariate correlation analysis demonstrated a significant association between time to maximum HR and BRS mean slope during recovery ($r = -0.579$; $p = 0.024$) and a trend towards association with BRS mean slope during tilt ($r = -0.457$; $p = 0.087$).

Sudomotor function assessment

Participants with AN demonstrated a trend towards decreased ESC in the hands ($p = 0.079$) and significantly decreased ESC in the feet, in comparison with both HCs ($p = 0.041$) and AN-WR ($p = 0.003$) (see Table 4.). Moreover, the AN group displayed significantly higher asymmetry between the ESC of the right and left feet than HCs ($p = 0.044$) and AN-WR groups ($p = 0.008$).

There was no significant difference in the CAN risk score between the groups, given the large variation within the AN group (range: 0–14). Within the AN group, univariate correlation analysis demonstrated a significant association between duration of AN and CAN-RS (Spearman's $r = -0.874$; $p < 0.001$). Four individuals with both increased duration of AN (19, 23, 25 and 34 years) and increased CAN risk scores (CAN-RS = 4, 5, 13, 14, respectively) drove this association.

Catecholamine outcomes

There were no differences in catecholamine and metabolite levels across the three groups (see Table 5).

Discussion

The current study provides a comprehensive assessment of ANS function in women with anorexia nervosa: in both the acute state and following weight restoration. Our primary hypothesis was partly supported: abnormalities in ANS profiles in patients with acute AN included bradycardia, decreased BP, decreased HRV, decreased BPV, decreased central nervous system sympathetic outflow to the skeletal musculature and decreased sudomotor function. However, our secondary hypothesis was not supported: individuals with a previous diagnosis of AN who were weight-restored also demonstrated decreased sympathetic outflow to the skeletal musculature as well as decreased BRS in response

Table 2 Heart rate variability, blood pressure variability and baroreflex sensitivity across groups

	AN M ± SD N = 10	AN-WR M ± SD N = 17	HC M ± SD N = 10	*F* or test statistic	*p* value
Heart rate variability					
SD rate (bpm)	2.61 ± 1.18	4.09 ± 1.35[b]	4.62 ± 1.30[a]	6.673	**0.004**
SDSD (ms)	47.6 ± 20.5	48.9 ± 32.0	56.4 ± 23.8	0.323	0.726
HF power (%)	40.5 ± 19.2	35.9 ± 14.2	33.2 ± 14.5	0.554	0.580
LF power (%)	22.0 ± 5.78	30.0 ± 12.4	29.6 ± 7.32	2.348	0.111
VLF power (%)	31.7 ± 22.6	32.2 ± 13.3	36.3 ± 11.4	0.268	0.767
LF/HF power (%)	0.77 ± 0.61	1.08 ± 0.68	1.11 ± 0.55	0.959	0.393
HF power (nu)	56.8 ± 11.5	52.1 ± 15.5	50.8 ± 13.7	0.516	0.602
LF power (nu)	35.9 ± 16.1	45.2 ± 18.0	47.7 ± 13.7	1.485	0.241
Blood pressure variability					
Resting SBP SD (mmHg)	0.041 ± 0.055	0.038 ± 0.021	0.087 ± 0.133[b]	6.179[d]	**0.046**
Resting DBP SD (mmHg)	0.028 ± 0.033	0.030 ± 0.015	0.068 ± 0.064[a,c]	9.430[d]	**0.009**
Tilt SBP SD (mmHg)	0.081 ± 0.056	0.133 ± 0.194	0.190 ± 0.334	0.880[d]	0.644
Tilt DBP SD (mmHg)	0.062 ± 0.055	0.085 ± 0.107	0.149 ± 0.188	2.280[d]	0.320
Recovery SBP SD (mmHg)	0.049 ± 0.044	0.043 ± 0.026	0.104 ± 0.168	1.832[d]	0.400
Recovery DBP SD (mmHg)	0.043 ± 0.051	0.039 ± 0.021	0.098 ± 0.117[a,c]	8.828[d]	**0.012**
Baroreflex sensitivity					
Resting mean slope (ms/mmHg)	59.41 ± 30.50	49.08 ± 46.92	56.08 ± 21.02	0.268	0.766
Resting BEI	29.41 ± 12.26	31.30 ± 15.30	37.45 ± 19.93	0.715	0.496
Tilt mean slope (ms/mmHg)	14.34 ± 11.49	9.14 ± 4.09	20.51 ± 10.92[c]	4.721	**0.016**
Tilt BEI	48.12 ± 14.37	47.64 ± 15.42	48.11 ± 9.66	0.005	0.995
Recovery mean slope (ms/mmHg)	55.54 ± 31.16	51.14 ± 32.93	69.25 ± 28.05	1.045	0.363
Recovery BEI	38.01 ± 14.91	34.93 ± 14.55	41.89 ± 12.93	0.721	0.494

Bold indicates a significant difference between the three groups

AN anorexia nervosa; *AN-WR* weight-restored; *HC* healthy controls; *BMI* body mass index; *M* mean; *SD* standard deviation; *SBP* systolic blood pressure; *DBP* diastolic blood pressure; *SD rate* standard deviation of the heart rate; *SDSD* standard deviation of the differences between successive heartbeat intervals; *HF* high-frequency; *LF* low frequency; *VLF* very low-frequency; *nu* normalised units; *BEI* baroreflex efficacy index

[a] $p < 0.01$; compared to AN

[b] $p < 0.05$; compared to AN

[c] $p < 0.05$; compared to AN-WR

[d] Kruskal–Wallis test statistic

Table 3 Head up tilt table test

	AN M ± SD N = 10	AN-WR M ± SD N = 17	HC M ± SD N = 10	*F* statistic	*p* value
Average increase in HR (bpm)	35 ± 12	36 ± 8	38 ± 6	0.452	0.640
Average SBP change (mmHg)	0.96 ± 2.40	2.18 ± 5.64	3.98 ± 5.86	0.836	0.444
Average DBP change (mmHg)	−5.64 ± 3.74	−5.76 ± 6.67	−4.24 ± 5.72	0.203	0.818
Max HR (bpm)	90.81 ± 18.49	103.25 ± 11.87	103.32 ± 8.79	3.110	0.058
Time to max HR (min:sec)	7:41 ± 2:25	5:44 ± 2:55[a]	8:27 ± .53	6.178[b]	**0.009**

Bold indicates a significant difference between the three groups

AN anorexia nervosa; *AN-WR* weight-restored; *HC* healthy controls; *BMI* body mass index; *M* mean; *SD* standard deviation; *HR* heart rate; *SBP* systolic blood pressure; *DBP* diastolic blood pressure

[a] $p < 0.01$; compared to HC

[b] Welch's *F*

Table 4 Sudoscan

	AN M ± SD N = 10	AN-WR M ± SD N = 17	HC M ± SD N = 10	*F* or test statistic	*p* value
Hands ESC (μS)	54.4 ± 24.24	67.65 ± 18.15	74.40 ± 16.57	2.737	0.079
Feet ESC (μS)	57.90 ± 24.34	82.41 ± 13.29[a]	77.50 ± 14.89[b]	6.546	**0.004**
Hand asymmetry (%)	14.80 ± 16.82	5.35 ± 6.02	3.10 ± 3.45	2.706[c]	0.094
Feet asymmetry (%)	11.50 ± 10.59	1.82 ± 2.27[a]	2.30 ± 2.41[b]	7.529[c]	**0.023**
CAN-RS	3.60 ± 5.54	0.35 ± 1.22	0.80 ± 1.93	3.618[c]	0.164

Bold indicates a significant difference between the three groups

AN anorexia nervosa; *AN-WR* weight-restored; *HC* healthy controls; *M* mean; *SD* standard deviation; *ESC* electrochemical skin conductance; *CAN-RS* cardiac autonomic neuropathy risk score

[a] $p < 0.01$; compared to AN

[b] $p < 0.05$; compared to AN

[c] Kruskal-Wallis test statistic

Table 5 Catecholamine outcomes

	AN M ± SD N = 8	AN-WR M ± SD N = 15	HC M ± SD N = 8	*F* statistic	*p* value
DHPG (pg/mL)	1289 ± 307	1155 ± 462	1236 ± 294	0.329	0.722
Noradrenaline (pg/mL)	452 ± 120	429 ± 116	442 ± 106	0.115	0.891
Adrenaline (pg/mL)	7 ± 3	10 ± 14	7 ± 8	1.294	0.290

AN anorexia nervosa; *AN-WR* weight-restored; *HC* healthy controls; *M* mean; *SD* standard deviation; *DHPG* dihydroxyphenylglycol

to a HUT, as compared to HCs, which are suggestive of some prolonged impact of starvation on ANS regulation.

Muscle sympathetic nerve activity

To the best of our knowledge, this is the first study to use a direct assessment of sympathetic nerve activity in individuals with a current or previous diagnosis of AN. MSNA was positively associated with BMI across the sample, demonstrating a gradient of increased MSNA across AN, AN-WR and HC groups. These results will be interpreted within the context of the current understanding of the physiological response to starvation, and implications will be proposed.

Previous studies have demonstrated that cellular metabolism is suppressed during starvation, with the suggestion that the body down-regulates its metabolic rate to preserve energy during starvation [57–59]. However, the specific factors that contribute to the down-regulation of cellular metabolism during starvation have been speculative. Abnormalities in glucose levels and regulation have been observed in individuals with AN; decreased blood glucose levels are common in AN [60] and were evident in the current sample, as reported previously [61]. In the general population, evidence has demonstrated that carbohydrate ingestion, including oral glucose, elicits increased sympathetic nervous system (SNS) activity (as assessed by regional rates of spillover of noradrenaline to plasma [62] and MSNA [63, 64]). Therefore, decreased glucose levels in AN may contribute to the depressed sympathetic activity seen in the current sample, providing a potential physiological basis for decreased energy expenditure and metabolism within skeletal muscle in the state of starvation. A previous investigation highlighted that postprandial glucose levels trended towards normal levels following 2 weeks of weight restoration, yet suggested that the long-term effects of starvation remained unclear [65]. The current study adds to this by demonstrating that decreased sympathetic activity continues for at least 12 months of weight restoration.

Another potential regulatory mechanism that may underlie the low sympathetic activity demonstrated in AN is leptin levels, which are demonstrably low in individuals with AN (for a review, see [66]). Of relevance to the current study, leptin modulates ANS and cardiovascular regulation through stimulation of sympathetic outflow [67]; thus low levels of leptin in AN may be an underlying regulatory mechanism of the decreased sympathetic activity in an effort to reduce energy expenditure and favour weight gain. However, leptin concentrations have been shown to normalise with refeeding prior to weight normalisation [66]; therefore, the rapid alterations in leptin levels in response to acute changes in

energy availability do not explain the decreased sympathetic activity in the weight-restored participants in the current study.

The haemodynamic implications of decreased MSNA in individuals with AN also provide a physiological mechanism for the prevalence of low BP and bradycardia in individuals with AN. Reduction in sympathetic tone has been demonstrated to lower BP [68]; therefore, the low BP seen in AN could be attributed, at least in part, to low sympathetic activity. Moreover, the predominance of vagal cardiovascular control likely underlies bradycardia in individuals with AN. While these haemodynamic abnormalities can be logically related to the decreased MSNA, a link between decreased sympathetic outflow and other cardiovascular complications and mortality seen in AN is less clear.

A major contributor to cardiovascular mortality is ventricular tachyarrhythmia, with evidence that patients who have recently recovered from a life-threatening ventricular arrhythmia exhibit substantially increased cardiac sympathetic activation as assessed from measures of the rate of noradrenaline spillover to plasma from the heart and the whole body [69]. This activation is thought to be a reflex response to reduced left ventricular function [69]. Moreover, significantly elevated total and cardiac sympathetic activity have been observed in patients with heart failure [70], which has a direct adverse effect on survival [71]. This association between increased sympathetic activity and poor prognosis in patients with heart failure has also been observed through MSNA recordings [72, 73]. While it remains uncertain whether increased sympathetic activation is causal, or a marker, of arrhythmias and heart failure, overactivity of the SNS is strongly associated with cardiovascular morbidity and mortality. Given that individuals with a current or previous diagnosis of AN do not demonstrate increased sympathetic activity, it is unlikely that the cardiovascular complications demonstrated by individuals with AN are due to increased SNS activity. Moreover, while other studies have demonstrated increased MSNA in individuals with depression [74] and anxiety [75], our results did not reflect this, despite high levels of depression and anxiety in the current sample [61], and in AN in general [76, 77]. While increased sympathetic activity was not seen in the participants with a current or previous diagnosis of AN in this study, bradyarrhythmia, caused by increased vagal tone and a concomitant decrease in sympathetic modulation [14], may be a contributing factor underlying the cardiovascular complications seen in AN and is an avenue that requires further investigation.

Catecholamines

The assessment of plasma adrenaline and noradrenaline and its intraneuronal metabolite, DHPG, revealed no differences among the three groups in the current study. This may reflect the dependence of plasma noradrenaline on not only the rate of noradrenaline release, but also rates of removal from plasma [78], thereby providing a poor indication of regional activity and an overall unreliable measure of sympathetic nerve activity [79].

Sudomotor function

In addition to decreased central SNS activity, as assessed by MSNA, individuals with a current diagnosis of AN demonstrated decreased sudomotor nerve activity, with an association between increased duration of AN and increased CAN risk within the AN group. Specifically, four individuals with a long duration of AN demonstrated increased CAN risk scores, suggesting that there may be substantial sudomotor dysfunction in individuals with long and enduring AN. Sudomotor function complements cardiovascular autonomic control by maintaining stable thermoregulation through dilation or constriction of cutaneous vessels and sweat production [80]. Previous studies have confirmed that people with AN have a significantly lower core body temperature [81], with adaptive mechanisms including the appearance of characteristic lanugo to retain heat [82]. The cholinergic (sudomotor) dysfunction demonstrated in individuals with AN may contribute to an inability to maintain thermal homeostasis while underweight through dysregulation of vascular control and circulation. Additional evidence to support the notion that decreased sudomotor activity may be a direct thermoregulatory response to reduced subcutaneous fat is the presence of decreased sudomotor function in cachexia secondary to cancer [83]. Furthermore, the significantly decreased ESC demonstrated by those with AN in the current study was not seen in those who were weight-restored, suggesting that sudomotor function returns alongside normalisation of weight.

Heart rate, blood pressure and heart rate variability

Replicating many previous findings, individuals in the acute stage of AN in this study demonstrated decreased HR and BP. In weight-restored participants, these haemodynamic parameters were similar to those in controls, further suggesting that decreased HR and BP reflect an adaptive response to energy deprivation. However, in contrast to a recent systematic review [16], we found decreased HRV in the AN group within the time domain, with no difference between individuals with AN and HCs in the frequency domains. There were no differences between AN-WR and HC groups in these parameters, consistent with a recent review [15]. Observations of HRV in AN have previously been highlighted as inconsistent [17], and our results provide further

evidence for the notion that HRV does not reflect the autonomic state of the entire body, but rather sinoatrial node regulation [84]. Specifically, HRV parameters may reflect parasympathetic activity, but interpretations of sympathetic activity from HRV remain questionable [18, 19], given our MSNA findings.

Blood pressure variability, cardiac baroreflex sensitivity and head-up tilt table test

While there were minimal differences in HRV across the three groups in our study, individuals with AN demonstrated significantly decreased variability in both systolic and diastolic BP at rest, suggesting increased parasympathetic control over the heart. Moreover, those who were weight-restored also showed decreased variability in diastolic BP. However, there was no difference between groups in BRS at rest, replicating the results of a recent investigation [22], but in contrast to previous reports [23, 24].

Similarly, there were no substantial differences between individuals with AN and HCs in response to the HUT. Having said this, three AN-WR participants experienced orthostatic intolerance shortly after beginning the HUT and the AN-WR group as a whole reached their peak HR during the tilt 2 min earlier than the AN group and almost 3 min earlier than HCs. Further investigation within the AN-WR group found that reaching peak HR earlier was associated with decreased BRS during recovery from tilt and a trend towards BRS during tilt. This suggests some degree of autonomic dysregulation is maintained following weight restoration.

Limitations

The primary limitation of the current study is the relatively small sample of people with a current diagnosis of AN. An addition limitation is the lack of definitive conclusion regarding the contributing mechanisms underlying the altered ANS activity. However, the current study provides a thorough assessment of ANS activity utilising multiple methodologies in both underweight and weight-restored participants with AN. The results of the current study do not generalise to males with AN.

Conclusion

The current study demonstrated that women with a current diagnosis of AN have significantly decreased sympathetic activity, which does not completely normalise following weight restoration and may reflect a physiological response to decreased energy intake. Moreover, while underweight, individuals with AN display a high risk of cardiac autonomic neuropathy, as assessed by sudomotor function, which increases alongside the duration of malnutrition. The indirect assessments of ANS function that were conducted in the current study also indicated decreased HRV in underweight participants and decreased BPV in both underweight and weight-restored participants. However, the AN-WR participants displayed some abnormal ANS function in response to a HUT that was not seen in the AN group. This may suggest that specific abnormalities in ANS function occur in the acute state of AN, whilst other abnormalities occur after the process of weight restoration. While it is unlikely that the cardiovascular complications seen in people with AN are due to high sympathetic activity, the consequences of sustained decreased activity and discordant ANS regulation are unknown and remain an avenue for future investigation. Future studies should also investigate direct assessment of sympathetic activity in long-term weight-restored participants in order to determine whether decreased activity is sustained or returns to normal.

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Availability of data and material The data sets used in the current study are available from the corresponding author on reasonable request.

Declarations

Conflict of interest The authors declare no conflict of interest for this research.

Ethical approval The research protocol was approved by the human research ethics committees at St Vincent's Hospital, Melbourne (HREC/18/SVHM/126), Swinburne University of Technology (SHR Project 2018/183) and The Melbourne Clinic (Project 307).

Consent to participate All participants provided written informed consent.

Consent for publication The manuscript does not contain any individual personal data in any form. All authors reviewed and approved the final version of the manuscript.

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