Autonomic Response to a Visceral Stressor is Dysregulated in Irritable Bowel Syndrome and Correlates with Duration of Disease

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Abstract

Background—Previous studies reported altered autonomic nervous system (ANS) responses in IBS at baseline and to colonic balloon distension. This study examined heart rate variability (HRV) and plasma catecholamines as an index of ANS responsiveness in IBS during flexible sigmoidoscopy (FS) and explored associations of HRV with clinical measures.

Methods—Rome III positive IBS patients and healthy controls completed questionnaires measuring GI and psychological symptoms. HRV measures were calculated using electrocardiogram (ECG) data at rest and during FS. Plasma catecholamines were measured before and after the FS. Linear mixed effects models were used to compare HRV with IBS status and IBS duration across six time points. Significance was assessed at the 0.05 level.

Results—36 IBS patients (53% F, mean age 37.89) and 31 controls (58% F, mean age 37.26) participated. After adjusting for age, sex, BMI, and HAD anxiety, IBS patients had a non-significant lower cardiovagal tone (p=0.436) and higher cardiosympathetic balance (p=0.316) at rest. During FS, controls showed a transient increase in cardiosympathetic balance and decrease in cardiovagal tone. However, IBS patients had significantly less cardiosympathetic and cardiovagal responsiveness both leading up to (p=0.003, p=0.005) and following (p=0.001, p=0.001) this stimulus. Those with longer duration of disease had less cardiosympathetic (p=0.014) and cardiovagal (p=0.009) responsiveness than those with shorter duration. No differences in catecholamines between IBS and controls were found.

Conclusion—IBS demonstrated dysregulated ANS responses to a visceral stressor which could be related to disease duration. Therefore, autonomic dysregulation is an objective physiologic correlate of IBS.

Keywords

autonomic dysregulation; autonomic nervous system; blunting; duration of disease; heart rate variability; irritable bowel syndrome; physiologic correlate; plasma catecholamines; somatic stressor; visceral stressor

Introduction

Irritable bowel syndrome (IBS) is a functional gastrointestinal (GI) disorder affecting 7–20% of the U.S. population, occurring twice as frequently in women than men (1, 2). It is characterized by chronic or recurrent abdominal pain with bowel habit changes, such as diarrhea and/or constipation (1, 3). Although its pathophysiology is not completely understood, IBS is considered a stress-sensitive disorder. Stress activates the hypothalamic-pituitary-adrenal (HPA) axis and the locus coeruleus-noradrenergic (LC-NE) systems (4). Dysregulations in basal and stress-induced activity of both the HPA axis and autonomic nervous system (ANS) have been demonstrated in IBS (5). The two branches of the ANS, the sympathetic nervous system (SNS) and parasympathetic nervous system (PNS), communicate with the enteric nervous system to modulate and coordinate functions such as GI motility, secretion, and immune functions (5–7), and thus ANS dysregulation may play an important role in IBS pathophysiology.

Various components of heart rate variability (HRV) can be used to index function of the SNS and PNS (8–11). While HRV is a measure of cardiac autonomic function it has been used as an indirect marker of central autonomic control and to some extent GI ANS function (12, 13). Relative power in high frequency (HF) represents cardiovagal, or cardiac parasympathetic activity. The ratio of low frequency power to HF power (LF/HF) represents cardiosympathetic balance (14–19).

Given the higher incidence of IBS in women compared to men, sex may have an effect on ANS function in IBS. However studies examining the effect of sex on ANS function in IBS and controls are scarce. Most studies include women only and those that include both sexes show inconsistent findings (13). We previously found that during rectosigmoid balloon placement and phasic sigmoid distensions, IBS men demonstrated greater ANS dysregulation than IBS women, who were not significantly different from healthy controls. IBS men had a higher cardiosympathetic and lower cardiovagal tone both before and during the visceral stressor than both IBS women and controls (16).

In addition to HRV, catecholamine levels including norepinephrine (NE) and epinephrine (EPI) can be used to assess SNS activity. Studies have shown higher levels of NE and EPI in IBS men and IBS women at baseline (20, 21) compared to controls however limited data is available with respect to visceral stimulation. In a previous study, we found significantly higher plasma NE levels in IBS vs. controls before and after rectal balloon distension associated with auditory stress but they did not change in response to the stress and distension conditions (22).

We have previously shown that the HPA axis is activated in response to a visceral stressor in IBS patients and healthy controls (23). The aims of the current study were to: 1) measure ANS function (i.e., cardioautonomic tone, plasma catecholamines) in IBS patients and healthy controls in response to a novel visceral stressor (flexible sigmoidoscopy [FS]), 2)

perform exploratory analyses to determine if ANS responses are associated with IBS characteristics (i.e., bowel habit, disease duration, IBS symptom severity), 3) determine how sex affects ANS response in IBS and controls.

Materials and Methods

Study Subjects and Recruitment

IBS patients 18–55 years of age who fulfilled Rome III diagnostic criteria (1, 24) were recruited from community advertisements. A gastroenterologist with expertise in IBS (LC) confirmed the diagnosis. Additionally, bowel habit subclassification was determined using Rome III criteria (24). IBS patients were excluded if they had a current history of a psychiatric disorder. Healthy controls were recruited by newspaper or internet advertisement from the community. Controls only had no history of IBS, other chronic pain conditions, or psychiatric illness, and were not taking beta-adrenergic blockers or centrally-acting drugs or those that could affect ANS function (anxiolytics, narcotics, antidepressants). (25) Subjects were excluded if they smoked more than 0.5 packages of cigarettes daily, had 400 mg caffeine daily (equivalent to a 16oz cup of standard-brew coffee), or exercised 1 hour or more per day. For premenopausal women not taking oral contraceptive agents or provera, menstrual cycle phase was determined by the count forward/backward method (menses: first three days of menses; follicular: days 4–14; luteal: day 14 - onset of menses). Women who took oral contraceptive agents were studied during the 4–14 days of their menstrual cycle, similar to the follicular phase. Plasma progesterone was collected at baseline (blood draw #1, see Table 1) to help confirm cycle phase. None of the post-menopausal women were taking hormone replacement therapy. Subjects were compensated for participating in the study. Informed consent was obtained from all subjects.

The study was approved by the University of California Los Angeles (UCLA) Institutional Review Board and was conducted in accordance with the institutional guidelines regulating human subjects research.

Symptom Measures

At the screening visit, a bowel symptom questionnaire was used to assess the presence and severity of IBS symptoms and duration of disease (26). Duration of disease is defined as current age minus the age of first onset of IBS symptoms. Current depression and anxiety symptoms were measured using the Hospital Anxiety and Depression (HAD) scale, a self-assessment scale utilized to detect current symptoms of depression and anxiety in subjects at baseline (27). Each subject also underwent a structured psychiatric interview (MINI) to measure past or current psychiatric disease (28).

At the study visit, a verbal descriptor anchored visual analog scale (VDVAS) (29) which assesses sensory intensity and unpleasantness of GI symptoms was administered prior to the procedure to measure the severity of IBS symptoms over past 24 hours and also immediately after the sigmoidoscopy to measure abdominal symptoms during the procedure.

Protocol

At the study visit, sigmoidoscopy to at least 40 cm from the anal verge was performed in the Medical Procedures Unit in the early afternoon (between 12 pm and 2 pm). Subjects used two tap-water enemas for bowel preparation. The 2-lead electrocardiogram (ECG) data was collected using the BioPac recording system (MP100A-CE, BioPac Inc., Santa Barbara, CA, USA) for 5-minute periods at 6 different time-points (see Table 1) before, during, and after the FS visceral stressor. All ECG measurements were taken in supine position with minimal movement. Two blood draws for catecholamine levels were performed, one at baseline

(blood draw #1) and one immediately following the sigmoidoscopy (blood draw #2). Plasma catecholamines (NE and EPI) were assayed at ARUP Laboratories (Salt Lake City, UT, USA) using high performance liquid chromatography. Additionally, sigmoid colonic mucosal biopsies were obtained after positioning of the sigmoidoscope for use in an associated study (30). If any signs of organic gastrointestinal disease were found on sigmoidoscopy, the subject was excluded from the study and referred to a clinician for further diagnostic evaluation.

Heart Rate Variability (HRV) Analysis

ECG data was manually screened for artifacts and the Acq*Knowledge* peak detection algorithm (V3.8.2, BioPac Inc., Santa Barbara, CA, USA) was used to determine the intervals between adjacent QRS complexes. The inter-beat (R-R) interval data was used for HRV analysis (Kubios HRV 2.0, Biosignal Analysis and Medical Imaging Group, Dept. of Physics, University of Kuopio, Finland). Autoregression (AR) frequency analysis and Fast Fourier transform (FFT) analysis was performed on one continuous 2-minute segment (epoch) of data in each 5-minute period in order to avoid inclusion of artifacts in the majority of samples, as any single artifact can distort HRV analysis (10). Epochs were chosen from the center of the period and were shifted, first downstream then upstream, to avoid artifacts when possible. The AR and FFT analysis yielded measures of high frequency (HF) power in normalized power based on a frequency band of 0.15–0.4 Hz and the LF/HF ratio with low frequency (LF) power defined as the interval between 0.04–0.15 Hz. A model order of 16 was used in the AR analysis with no spectral factorization. FFT analysis utilized Welch's periodogram method with a window width of 256 seconds and window overlap of 50%.

Our primary measures of HRV are based on the AR approach, which takes the data as a composite of deterministic and stochastic components and utilizes a best-fit model to compute the spectral values. AR analysis excludes "noise" by concentrating on more significant data points, allowing for improved resolution compared to FFT analysis, which treats all data as deterministic components (19). However, much of the HRV literature utilizes FFT analysis as it is considered more descriptive (19) so in a secondary analysis in this study, we performed FFT spectral analysis on the interbeat interval data and compared the results to the AR analysis to allow for comparison with previous HRV studies as well as demonstrate the robustness of our findings.

Statistical analysis

Descriptive statistics of baseline measures were summarized for categorical (frequency [%]) and continuous (mean±SE) variables. The Kruskal-Wallis and Fisher's exact test were utilized to test group differences among continuous variables and categorical variables, respectively. Spearman's correlation was used to assess the association between continuous variables. Linear mixed effects models were used to compare HRV values measured across six time points (Table 1) to the interaction of IBS status with time while controlling for clinical traits (gender, age, BMI and HAD anxiety). Time plots indicated that there was a change in slope after the FS and, therefore time was modeled such that the rate of change (slope 1) from the rest period to the FS was allowed to differ from the FS to blood draw #2 (slope 2) for the IBS and control comparisons. The interaction of sex and disease status (IBS, controls) on ANS measures was also evaluated using linear mixed models. To examine the effect of IBS duration on ANS responses, a linear time model was assumed. We also evaluated the association of change in ANS measures over time with IBS symptoms (current intensity and unpleasantness over past 24 hours, intensity and unpleasantness of the FS procedure, and duration of disease). IBS duration was coded as a continuous variable while controlling for the same clinical traits. For the linear mixed effects models, non-

Gaussian variables were log transformed to achieve approximate normality. Changes in ANS responses were depicted in figures using a LOESS function which smoothes out the individual fluctuations at each time period for better visualization of patterns over time. All analyses were performed using SAS version 9.3 (SAS Institute, Cary, NC, USA) or R version 2.15.1 and significance was assessed at the 0.05 level.

Results

Baseline Clinical Characteristics

All baseline clinical characteristics of the subjects are summarized in Table 2 and are evaluated using a Kruskal-Wallis or Fisher's exact test as appropriate. The 67 subjects included 36 IBS patients (19 women and 17 men) and 31 healthy controls (18 women and 13 men). The three main bowel habit subtypes were similarly represented within the IBS group. There were no significant differences in menstrual cycle phase distribution between IBS and control women. IBS patients did not differ significantly from controls in mean age, however age did significantly affect our HRV in our models, a finding previously reported in literature (31, 32), and was controlled for in our analysis. IBS patients and controls did not significantly differ in mean body mass index (BMI).

Compared to healthy controls, IBS patients had significantly higher mean HAD anxiety and depression symptom scores, but the majority of the anxiety (75%) and depression (92%) scores for IBS patients were within either the non-case (0–7) or doubtful case (8–10) range (27) and all of the anxiety and depression scores for controls were within the non-case or doubtful case range. Within the IBS group, only 9 subjects had anxiety scores in the definite case range of 11 or greater and only 3 had depression scores in this range. Neither HAD anxiety nor depression scores significantly affected the ANS responses. Compared to controls, IBS patients rated their abdominal symptoms during the FS procedure as significantly more unpleasant (9.5 vs. 5.67, p<0.001) and intense (11.46 vs. 6.15, p<0.001), but these ratings did not correlate with ANS responses.

None of the IBS patients were taking beta-adrenergic blockers and the majority (86%) of IBS patients were not currently taking centrally acting drugs or those that could affect ANS function. Only one IBS subject was taking an antidepressant medication (i.e., SSRI) at the time of the study.

HRV in IBS vs. Controls

One healthy control man was excluded due to irregular cardiac rhythm which interfered with the HRV analysis. More than 90% of subjects had usable HRV data for all periods except blood draw #2, where only 64% of subjects had usable HRV data (Table 3). Missing period data are accounted for by ECG lead detachment or malfunction (21 out of 402 data points, 5.2%), computer malfunction (4 data points, 1.0%), movement artifact (3 data points, 0.7%), and procedural error (3 data points, 0.7%).

Baseline HRV Measures—After adjusting for age, gender, HAD anxiety, and BMI, HF power (cardiovagal tone, p = 0.436, Fig. 1) and LF/HF (cardiosympathetic balance, p = 0.316, Fig. 2) was similar in IBS patients and controls.

ANS Response to the FS procedure—During the baseline period and blood draw #1 prior to the FS procedure controls showed a decrease in HF power (i.e., cardiovagal tone) and increase in LF/HF (i.e., cardiosympathetic balance), while IBS patients had significantly less change in both HF power (p=0.005) and LF/HF (p=0.003) (Fig. 1–2). This pattern was seen again following the FS. As controls showed an increase in HF power and decrease in

LF/HF after FS, IBS showed significantly less change in both HF power (p=0.001) and LF/HF (p=0.001) (Fig. 1–2). Of note, differences in the mean values of these measures were statistically significant during the FS procedure (Supplemental Table 1).

Correlation between HRV and clinical characteristics

Sex differences—Women had significantly higher HF power (p = 0.008) and lower LF/HF (p=0.002) compared to men throughout all periods (Fig. 3A–B, Supplemental Table 2). Both men and women demonstrated the same trend in ANS measures across periods with a decrease in HF power (cardiovagal, p=0.001) and increase in LF/HF (cardiosympathetic balance, p<0.001) leading up to the FS procedure. Despite overall sex differences, there was no significant effect of sex on the ANS findings when comparing IBS patients and controls both prior to and after the FS.

Duration of Disease—While IBS patients with a shorter duration of disease demonstrated a significant decrease in HF power across all periods, those with a longer duration of disease had significantly less change in HF power throughout the study (p=0.009, Supplemental Table 3). Similarly, while IBS patients with a shorter duration of disease showed a significant increase in LF/HF across all periods, patients with IBS for a longer duration of time had significantly less change in LF/HF (p=0.014, Supplemental Table 3). In Fig. 4–5, we presented the duration of disease as a categorical variable of longer and shorter duration using the median split with longer duration   9 years (mean age 40.0 ±2.3yrs) and shorter duration < 9 years (mean age 36.6±1.5).

Current IBS Symptom Severity—Current IBS symptom unpleasantness and intensity scores in the past 24 hours of the study did not significantly affect the ANS responses in IBS vs. controls.

IBS Bowel Habit Subtype—ANS response did not significantly differ between the IBS bowel habit subgroups.

HRV using FFT Analysis instead of AR Analysis

When comparing the AR models to FFT models, all of our findings were in the same direction, but some of the interaction terms only reached borderline significance. IBS demonstrated less change in both HF power and LF/HF compared to controls, both prior to the FS (p=0.087 and p=0.096, respectively) and after the FS (p=0.027 and p=0.022, respectively). The sex effect was upheld with women having higher HF power and lower LF/HF (p=0.002 and p=0.003, respectively) compared to men across all periods with no significant difference in the rate of change, or trend across periods, between men and women for either HF power (p=0.133) or LF power (p=0.384). Lastly IBS subjects with longer duration of disease had significantly less change in HF power and LF/HF throughout compared to controls (p = 0.029 and p=0.015 respectively).

Catecholamine levels in IBS vs. Controls

Of the total number of subjects, 22 (61%; 13 are women) of the IBS patients and 21 (70%; 12 are women) of the healthy controls had catecholamine data both at baseline (blood draw #1) and following the FS (blood draw #2). Two IBS patients had catecholamine data at baseline only. All missing catecholamine data was due to the inability to obtain sufficient plasma samples for catecholamine analysis from the subject during the blood draws. IBS patients and healthy controls did not show significant differences at baseline for NE (mean ± SE: 275±22 vs. 247±20 pg/mL, p= 0.475) or EPI (24±4 vs. 28±5 pg/mL, p= 0.569). Similarly there were no differences in catecholamines for IBS vs. controls following the FS

in terms of NE (303±27 vs. 294±28 pg/mL, p=0.915) or EPI (26 ±5 vs. 29±4 pg/mL, p= 0.349). There were no significant changes in catecholamines from blood draw #1 to blood draw #2 for either IBS or controls and there were no sex differences.

Discussion

The main study results are: 1) IBS patients showed a significant blunting of ANS response to FS which was not seen in controls, 2) this blunting became more prominent with longer disease duration, and 3) Overall, women had higher cardiovagal tone and lower cardiosympathetic balance compared to men across all time points. The lack of difference in ANS measures between IBS and controls at baseline differs from most previous HRV analysis findings which show IBS patients having higher cardiosympathetic tone and/or lower cardiovagal tone compared to healthy controls (6, 33). Our main finding of ANS blunting in IBS in response to visceral stressor differs from the literature which generally shows greater cardiosympathetic activity in IBS patients during and after both rectal/colonic distensions (16) and meals (12, 34) compared to controls.

Our study is novel in several ways: 1) a standard GI procedure in clinical practice, FS, served as the visceral stressor, 2) the specific finding of ANS blunting across multiple distinct periods of time in IBS patients has not been previously reported, 3) an association of ANS and duration of disease has not been reported in the past in IBS, and 4) the sex difference in ANS may not be specific to IBS.

There are two previous studies which examined cardioautonomic tone across multiple time points including during rectosigmoid balloon distension. Both demonstrate significant dysregulation in ANS response to visceral stressor however the specific dysregulation patterns differ from the ones reported in this study. In one study, we conducted HRV analysis in 40 IBS patients and 16 controls to measure response to a rectosigmoid balloon distension and found that IBS had higher cardiosympathetic balance and lower cardiovagal tone than controls at all 3 time points: baseline, after balloon was positioned in colon, and during phasic sigmoid balloon distension (16). Additionally we discovered that IBS men had greater cardiosympathetic balance and lower cardiovagal tone compared to IBS women across all time points. In the second study, Ng et al. (2007) measured HRV in 8 IBS patients (mean age 39 yrs, 87.5% women; bowel habit 75% diarrhea, 25% constipation) and 8 healthy controls (mean age 38 yrs; 87.5% women) in response to colonic distension in both fasting and then fed states (1000 kcal liquid meal given 60 minutes after distension protocol) (34). They found no difference in cardiovagal tone between IBS and controls at any of the four time points: pre-distension fasting, during distension fasting, pre-distension fed, and during distension fed. IBS had significantly greater cardiosympathetic balance than controls in the pre-distension fed state only. In response to feeding, IBS patients demonstrated a significant decrease in cardiovagal tone and an increase in cardiosympathetic balance while controls showed no significant change in either measure. The only significant response to a visceral stressor was in IBS patients who demonstrated a decrease in cardiosympathetic tone only in the post-prandial state (34). Of note, while other studies may have performed sigmoidoscopies to place colonic balloons for distension, our current study differs in that we measured ANS measures immediately before, during and after the sigmoidoscopy, which also included collection of colonic mucosal biopsies. However, the biopsies were collected within a few minutes and did not cause additional abdominal discomfort or pain. Nonetheless, it is possible that the addition of colonic biopsies could have contributed to the ANS differences between our study's findings and that of previous ones.

The phenomenon of ANS blunting, though novel to functional gastrointestinal disorders, has been reported in chronic somatic pain syndromes. A previous study conducted in patients

with chronic pelvic pain syndrome (CPPS) showed that men with CPPS had no significant change in HF power or cardiovagal tone upon standing compared to controls (35). Another study conducted several years later showed that individuals with somatoform disorders have less cardiovagal reactivity during emotional stimuli compared to controls (36). In addition, a pediatric study showed that children with chronic fatigue syndrome had relatively unchanged cardiovagal and cardiosympathetic tone in response to head-up-tilt compared to both a healthy control group and patients evaluated for syncope (37). The occurrence of ANS blunting in both IBS and chronic pain syndromes suggests a shared pathophysiology involving dysregulation of the ANS. This idea is further supported by a 2010 population study in Minnesota, which found that of 129 women with pelvic pain, 7% met diagnostic criteria for IBS, demonstrating a significant overlap between the two diseases (38). The blunted ANS response to a visceral stressor in IBS suggests that autonomic dysregulation may extend to the interactions between the enteric nervous system and central nervous system within the brain-gut axis (39, 40).

Our study's finding that ANS blunting in IBS patients became more pronounced with duration of disease further supports the hypothesis that ANS blunting is a physiologic correlate of the "wear and tear" of the ANS over time in IBS. One theoretical confounder is the effect of age on ANS dysregulation, however our findings were still significant after controlling for age. An additional possible confounder is that IBS patients may have had greater past exposures to the flexible sigmoidoscopy and thus may demonstrate a blunted ANS response to the visceral stimulus as they are "familiar" with the procedure compared to healthy controls. However, in our study, the number of subjects with previous experiences of FS was comparable between the IBS and control groups (4 vs. 2 subjects). Additionally, subjects in the IBS group demonstrated significantly higher unpleasantness and intensity ratings of the procedure compared to controls, and thus this hypothesis of familiarity with the visceral stimulus is less likely.

The progressive degradation of ANS responsiveness over time has never been reported in GI functional disorders or other chronic pain disorders according to our knowledge, and hypotheses to explain the mechanism are scarce. Interestingly, the degree of ANS dysregulation showed no correlation with bowel habit type or the reported symptom severity, which differs from a recent study of 103 IBS and 49 healthy control women which showed significant ANS dysregulation only among IBS subjects with symptoms rated as severe and differing patterns of ANS derangement based on specific bowel habit subtype (17). Of note our study measured only current symptom severity and not severity over a prolonged period of time. Nonetheless, our study suggests progressive ANS dysregulation may be particularly affected by duration of disease and less so by IBS severity or symptom subtype.

Another possible confounder to our study is the ANS response to the nociceptive pain from the needle stick in blood draw #1 and blood draw #2. Somatic pain as a stressor in healthy subjects is known to activate the sympathetic nervous system which in turn can facilitate ANS descending inhibition of the nociceptive transmission (41). In IBS patients however, the response to somatic pain can differ significantly, particularly if they do not have a coexistent chronic somatic pain disorder. A study by Tousignant-Laflamme et al. (2006) which evaluated the ANS response to pain with a cold water immersion test in 14 IBS women and 13 healthy control women showed that the IBS patients had an ANS response to pain which was opposite of expected from healthy controls. IBS patients showed an increase in cardiovagal tone and decrease in cardiosympathetic balance in response to pain from cold water immersion (42). This ANS response to a somatic stimulus is opposite to that of a visceral stimulus and may be due to the fact that IBS patients without a known coexistent somatic pain disorder have a greater somatic pain tolerance to this particular stimulus

compared to healthy controls, suggestive of somatic hypoalgesia (43, 44). Nevertheless, our analysis did not find any significant change in either HF power or LF/HF attributable to blood draw #1 or blood draw #2 alone. The nociceptive pain from the blood draws may be contributing to the overall effect of the visceral stressor on the ANS response, however it is difficult to decipher based on the study's experimental protocol.

Another key finding of this study is that women had lower cardiosympathetic balance and higher cardiovagal tone compared to men throughout most time points. This sex difference in ANS activity is consistent with the literature which shows women having lower cardiosympathetic tone and/or higher cardiovagal tone at baseline than men in both healthy populations (45, 46) and IBS populations (16). No clear mechanism has been suggested to explain this overall ANS sex difference and studies looking at the relationship between the ANS disparities and hormonal differences frequently report conflicting and ultimately inconclusive results (46). In this study, we found that there was no sex effect on ANS differences between IBS and controls. This lack of sex effect on cardioautonomic activity in IBS suggests that autonomic dysregulation alone is not a predominant mechanism to explain the higher prevalence of IBS in women compared to men and reinforces the multifactorial etiology of IBS.

The possible weakness of this study is that blood levels for catecholamines could only be collected in about two-thirds of subjects. Therefore, failure to determine group and condition differences in catecholamines, such as the utility of plasma catecholamines as a gauge of SNS function, could be due to low statistical power.

In summary, we found that IBS patients had ANS dysregulation prior to, during and after a visceral stressor; and this autonomic dysregulation was more pronounced in patients with greater duration of disease suggestive of progressive autonomic dysfunction over time. The ANS dysregulation was not attributed to current symptom severity, bowel habit or sex. Further research is needed to elucidate the mechanism of this ANS dysregulation and integrate it into the understanding of the pathophysiology of IBS.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

Abbreviations

ANS	autonomic nervous system
AR	autoregression
BD	blood draw
EPI	epinephrine
FFT	fast Fourier transform

FS	flexible sigmoidoscopy
GI	gastrointestinal
HADS	hospital anxiety and depression scale
HF	high frequency
HRV	heart rate variability
IBS	irritable bowel syndrome
LF	low frequency
NE	norepinephrine
n.u.	normalized units
PNS	parasympathetic nervous system
SNS	sympathetic nervous system
VDVAS	Verbal Descriptor Visual Analog Scales

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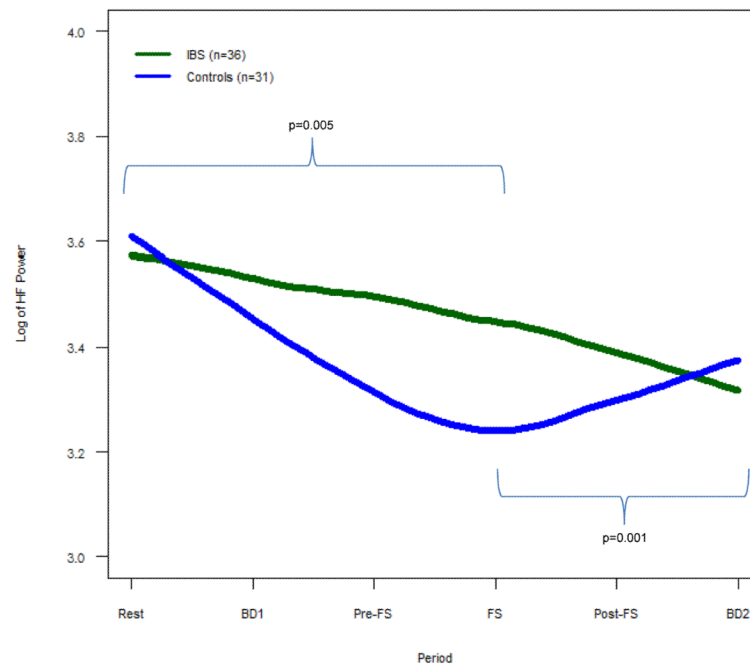
Figure 1. HF Power for IBS vs. Controls
Mean HF power, representing cardiovagal tone, is shown. IBS subjects demonstrate less change in HF power both leading up to FS (p = 0.005) and following FS (p=0.001) compared to controls.
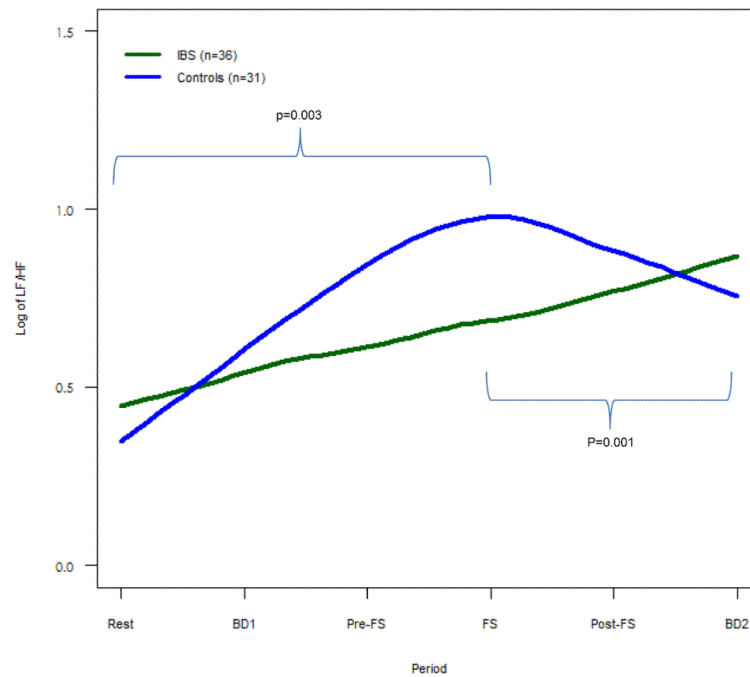
Figure 2. LF/HF for IBS vs. Controls
Mean LF/HF, representing cardiosympathetic balance, is shown. IBS subjects demonstrate less change in LF/HF both leading up to FS (p = 0.003) and following FS (p=0.001) compared to controls.
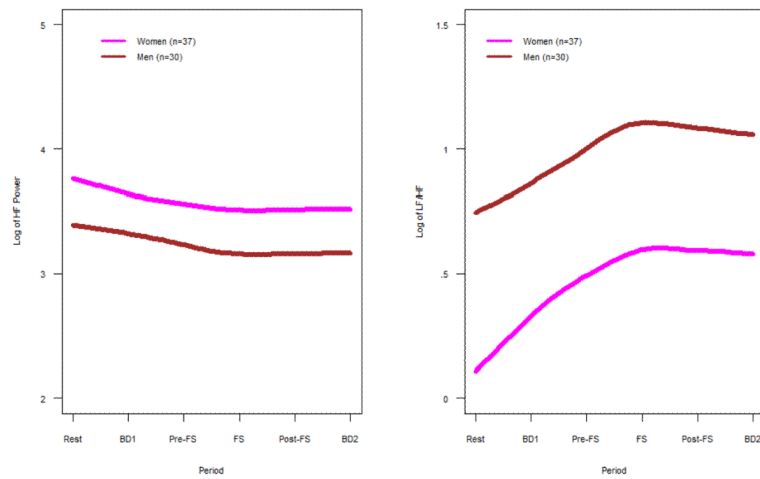
Figure 3.
A. HF Power for Women vs. Men: Mean HF power, which represents cardiovagal tone, is shown for women vs. men for all subjects. Women had significantly higher HF power across all periods compared to men (p = 0.008).
B. LF/HF Power for Women vs. Men: Mean LF/HF, which represents cardiosympathetic balance, is shown for women vs. men for all subjects. Women had significantly lower LF/HF across all periods compared to men (p = 0.002).
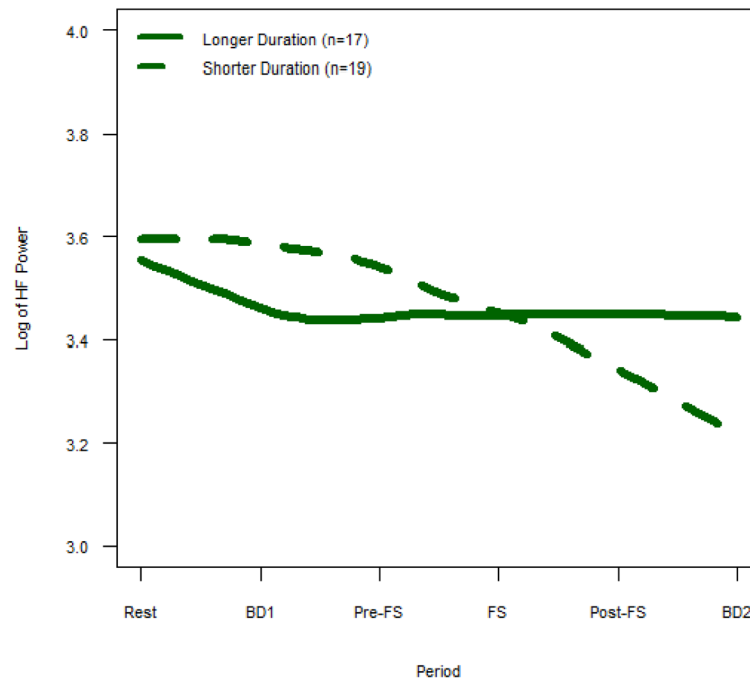
Figure 4. HF Power for Longer Duration vs. Shorter Duration IBS
Mean HF power, which represents cardiovagal tone, is shown for IBS subjects with longer vs. shorter duration of disease. Longer duration IBS subjects demonstrate less change in HF power across all periods compared to shorter duration IBS (p = 0.009).
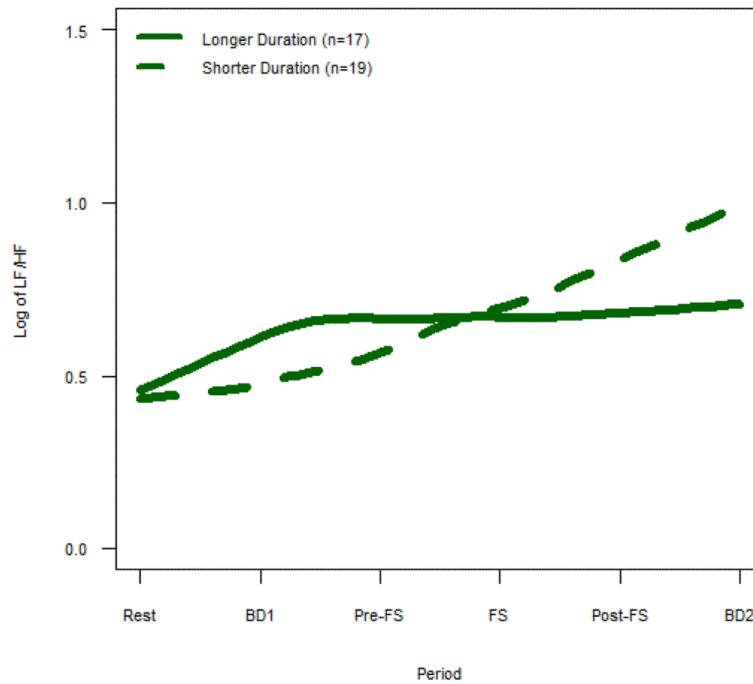
Figure 5. LF/HF Power for Longer Duration vs. Shorter Duration IBS
Mean LF/HF, which represents cardiosympathetic balance, is shown for IBS subjects with longer vs. shorter duration of disease. Longer duration IBS subjects demonstrate less change in LF/HF across all periods compared to shorter duration IBS (p = 0.014).

Table 1

Periods for Data Acquisition

Period*	Description
Rest	Baseline, patient lying still supine for 15 minutes before data collection
BD #1	Blood draw #1, data collected at start of venipuncture
Pre-FS	Period prior to starting sigmoidoscopy, patient in procedure room lying still supine for 15 minutes before data collection
FS	Insertion of flexible sigmoidoscope and position at 40 cm from anal verge, data collected when sigmoidoscope first reaches appropriate location (total duration of FS ~5–10 minutes)
Post-FS	Immediately after sigmoidoscope removal from anus
BD #2	Blood draw #2, data collected at start of venipuncture

Abbreviations: FS= flexible sigmoidoscopy, BD= blood draw

*
5 minutes of ECG data collected for each period

Table 2

Subject Characteristics

	IBS Patients (n=36)	Controls (n=31)	p-value
Age (Years±SE)	37.89±1.79	37.26±1.83	0.744
BMI (kg/m²±SE) *	26.83±1.00	25.18±0.80	0.299
Women (%)	19 (53%)	18 (58%)	0.810
Menstrual Cycle Phase Distribution for Women			0.498
Menses	1	0	
Luteal Phase	3	2	
Follicular Phase	10	14	
Peri-menopausal	1	1	
Menopausal	4	1	
HAD Scores (0–20±SE)			
Anxiety	6.42±0.90	2.55±0.4	0.002
Depression	3.25±0.62	0.90±0.27	0.005
VDVAS Current Symptom Severity Scores (0–20±SE) *			
Abdominal Symptoms Unpleasantness Rating Past 24 hours (IBS only)	7.64±0.52	NA	
Abdominal Symptoms Intensity Rating Past 24 hours (IBS only)	9.68±0.7	NA	
Unpleasantness Rating of Abdominal Symptoms During the Procedure	9.5±0.58	5.67±0.56	<0.001
Intensity Rating of Abdominal Symptoms During the Procedure	11.46±0.74	6.15±0.87	<0.001
Bowel Habits (%)			
Constipation	11 (31%)	NA	
Diarrhea	14 (39%)	NA	
Mixed/Alternating	10 (28%)	NA	
Unspecified	1 (2%)	NA	
Duration of IBS (Years±SE)	10.88 ± 1.48	NA	
Duration of IBS - Constipation Subtype	9.7±2.62	NA	
Duration of IBS - Diarrhea Subtype	10 ± 2.16	NA	
Duration of IBS - Mixed/Alternating Subtype	12.9±3.35	NA	
Duration of IBS - Unspecified Subtype	13	NA	

Abbreviations: HAD = Hospital Anxiety and Depression scale (27); VDVAS = Verbal Descriptor Visual Analog Scales (29); BMI = body mass index; IBS = irritable bowel syndrome

*
BMI not collected for 2 IBS subjects

**
VDVAS score not taken for 1 control subject.

Table 3

Number of Patients Who Had ANS Data for Each Period

Period	IBS Patients (n=36)	Healthy Controls (n=31)	Total
Rest	34	26	60
BD #1	35	30	65
Pre-FS	34	28	62
FS	34	30	64
Post-FS	31	29	60
BD #2	22	21	43 [*]

Abbreviations: FS= flexible sigmoidoscopy, BD= blood draw

See Table 1 for period descriptions.

[*] Twenty-four subjects missing data for blood draw #2: blood draw #2 was not attempted in 16 of 67 subjects (24%) when blood samples from the subject could not be obtained during blood draw #1, ECG leads detached or malfunctioned in 3 subjects (4.5%), there was a procedure error for 3 subjects (4.5%), 1 subject became hypovolemic in blood draw, and the computer system malfunctioned during the procedure for 1 subject.