STRESS REACTIVITY AND PHYSICAL ACTIVITY IN IBS

INVESTIGATING THE PROTECTIVE EFFECTS OF PHYSICAL ACTIVITY ON ACUTE STRESS REACTIVITY IN IBS PATIENTS

By EMMA NICHOLSON, B.Sc.

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TITLE: Investigating the protective effects of physical activity on acute stress reactivity in IBS patients

AUTHOR: Emma Nicholson, B.Sc. Honours Biochemistry (McMaster University)

SUPERVISOR: Dr. Jennifer J. Heisz, Ph.D.

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ABSTRACT

Introduction: Irritable bowel syndrome (IBS) is characterized by gastrointestinal (GI) symptoms, and as a consequence of dysregulated communication via the gut-brain axis, is highly comorbid with mental illnesses such as anxiety and depression. With no known cure, IBS patients must manage their symptoms through lifestyle factors. Physical activity is one such lifestyle factor that reduces GI symptoms and improves mental health; however, it remains unclear whether physical activity buffers against the acute worsening of IBS symptoms following a stressor.

Method: To investigate this, we evaluated the stress reactivity and recovery of 9 IBS patients and 13 healthy controls following exposure to acute stress. We exposed participants to an electronic Trier Social Stress Test (e-TSST) and measured changes in psychological stress (state anxiety), physiological stress (sympathovagal balance, where higher LF/HF ratio indicates greater stress system activation), and GI symptom severity before, during and every 20 minutes for one hour after. Physical activity was measured using the Stanford Seven-Day Physical Activity Recall questionnaire and quantified as weekly energy expenditure.

Results: IBS patients had higher state anxiety ($p = .05$), LF/HF ratio ($p = .01$) and GI symptom severity ($p = .01$) than healthy controls. Although the e-TSST did not exacerbate these group differences, higher state anxiety at baseline ($p = .03$) and higher LF/HF ratio in response to an acute stressor ($p < .001$) were associated with more severe GI symptoms within the first 20 minutes following the e-TSST. Importantly, IBS patients who were more physically active experienced less severe GI symptoms during that same timeframe ($p = .03$).

Conclusion: Physical activity may be a promising lifestyle factor for lessening GI symptom severity in response to an acute stressor.

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7DPAR	Stanford seven-day physical activity recall
ACTH	Adrenocorticotropic hormone
ANS	Autonomic nervous system
BDI-II	Beck depression inventory-II
CRF	Corticotrophin-releasing factor
ECG	Electrocardiogram
EM	Expectation-maximization
e-TSST	Electronic trier social stress test
GI	Gastrointestinal
GRs	Glucocorticoid receptors
HF	High frequency
HPA	Hypothalamic-pituitary-adrenal
HR	Heart rate
HRV	Heart rate variability
IBS	Irritable bowel syndrome
IBS-SSS	Irritable bowel syndrome symptom severity score
IDO	Indoleamine-2,3-dioxygenase
IL-6	Interleukin-6
LF	Low frequency
LF/HF	Low frequency to high frequency ratio
MET	Metabolic equivalent of the task
PNS	Parasympathetic nervous system
PPG	Photoplethysmography
PSS	Perceived stress scale
PSQI	Pittsburgh sleep quality index
SNS	Sympathetic nervous system
SONA	Psychology neuroscience and behaviour research participation system
STAI-6	State-trait anxiety inventory short form
TSST	Trier social stress test

DECLARATIONS OF ACADEMIC ACHIEVEMENT

Emma Nicholson's role:

- Developed initial ethics submission and amended application
- Designed study protocol and selected measures
- Recruited and screened participants
- Scheduled virtual visits and prepared online materials
- Trained and supervised undergraduate students who participated in virtual visit
- Led data collection, analysis, and interpretation
- Prepared manuscript

Role of co-authors:

- JH obtained study funding
- JH assisted EN with ethics submission and amendment
- JH assisted EN with study design and selection of measures
- JH assisted EN with data analysis and interpretation

INTRODUCTION

Irritable bowel syndrome (IBS) is a chronic condition that affects the large intestine and is characterized by abdominal cramping, pain, bloating, gas, and diarrhea or constipation (Lacy et al., 2016). The disorder is heterogeneous, including three distinct subtypes depending on the key symptomology: IBS-C (constipation), IBS-D (diarrhea) and IBS-M (mixed). Meta-analyses have revealed that IBS is more prevalent in women than men, and those under the age of 50 are at higher risk of IBS (Lovell & Ford, 2012; Mulak et al., 2014; Palsson et al., 2003). The higher risk in females may be related to the inverse relationship exists between female sex hormones, estrogen and progesterone, and IBS symptoms (Videlock et al., 2016); though it remains unclear whether symptoms worsen for a particular woman with IBS at different phases in her menstrual cycles (Houghton et al., 2002) (Videlock et al., 2016).

Given the heterogeneous nature of the disorder, its etiology also remains unclear. Various lifestyle factors have been linked to IBS risk and severity, including diet and early life stress. The impact of diet on IBS is related to its direct effects on the microbiome and downstream inflammation (Dalton et al., 2019; Shahar et al., 2020). Probiotics, diets and other lifestyle factors that promote microbial diversity and lower inflammation have been shown to improve IBS symptoms (Amirani et al., 2020; Böhn et al., 2015). Whereas early life stressors, such as physical and emotional abuse or neglect during childhood, have been implicated in the development of this syndrome given its sweeping effects on both the microbiome and one's stress reactivity (O'Mahony et al., 2009). Catecholamines, which are chemical signals released in the body during times of psychological distress, impact the gut motility by changing microbiota diversity (Lyte et al., 2011). The dual effects of IBS development on the stress system and gut

pathology highlight the gut-brain nature of the disorder and help to explain the high comorbidity of physical and mental distress in IBS patients.

Approximately 40-60% of individuals with IBS also experience anxiety disorders and depression (GIS, 2018), suggesting that communication between the gut and the brain is disrupted. About 70% of Canadians with IBS indicate their physical and psychological symptoms interfere with their everyday activities, and nearly half report having to miss school or work due to IBS (GIS, 2016). Therefore, there is an urgent need for interventions that mitigate IBS symptoms.

Although biological and environmental factors contribute to the symptoms and severity of IBS, a hyperactive stress response is a key biological factor implicated in IBS development that has the potential to give rise to both its physical and psychological symptoms. The stress response can be divided into the fast-acting sympathetic-adreno-medullar (SAM) axis and the slower acting hypothalamic-pituitary-adrenal (HPA) axis. When a threat or stressor is detected, a signal is sent to the hypothalamus which activates both the SAM and HPA axes. In the SAM axis, the hypothalamus signals the adrenal medulla to secrete catecholamines, epinephrine and norepinephrine. These catecholamines act on the sympathetic nervous system, preparing the body for fight or flight. Immediate increases in epinephrine and norepinephrine elevate heart rate and blood pressure, providing the body with immediate energy to deal with the stressor.

The HPA axis is also set in motion upon detection of a stressor, however, given it consists of a three-step hormonal cascade as opposed to direct stimulation like the SAM axis, its mobilization is slower. The HPA axis begins at the hypothalamus with the release of corticotrophin-releasing factor (CRF), which stimulates the pituitary gland to release adrenocorticotropic hormone (ACTH) and ends by stimulating the adrenal glands to release

cortisol. The longer the HPA axis is "on", the more cortisol is released into the blood. Rising cortisol increases blood glucose to provide the body with the essential energy it needs to maintain the sympathetic nervous system in a state of fight or flight, initially set into motion by the SAM axis. Stress reactivity is often indexed using psychological measures of state anxiety and physiological measures of catecholamines, cortisol and heart rate (HR), wherein higher levels of each variable indicate greater stress reactivity (Turner et al., 2020).

Once the threat is gone or a stressor has passed, the body turns "off" both the SAM and HPA axes. The parasympathetic nervous system, mainly the vagus nerve, counteracts the effects of SAM activation. Whereas a negative feedback mechanism involving rising cortisol "turns off" the HPA axis to inhibit further cortisol production. Specifically, the negative feedback mechanism is initiated when cortisol binds to glucocorticoid receptors (GRs) in the hypothalamus and pituitary gland. This inhibits the release of CRF and ACTH and halts the production of cortisol; thus, turning "off" or deactivating the HPA axis and its stress response. The speed at which both axis are turned off reflects how quickly one recovers from a stressor. This recovery is often measured by the rate at which physiological (catecholamines, HR, cortisol) and psychological (state anxiety) variables return to baseline. Poorer stress recovery, which is a key component of a hyperactive stress response, is characterized by a slower rate of return to baseline.

Given the links between dysregulated gut-brain communication and stress reactivity, it should come as no surprise that IBS patients have poor stress recovery. IBS patients have elevated norepinephrine (Heitkemper et al., 1996) indicative of sustained SAM axis activation that stems from suppressed vagus nerve activity (Liu et al., 2013; Salvioli et al., 2015). The HPA axis is overreactive and slower to recover in IBS patients because they lack sufficient GRs

needed to bind to cortisol to initiate the negative feedback mechanism (Videlock et al., 2016). This makes it harder for IBS patients to turn "off" their stress response, leaving their psychological and physiological stress systems constantly active, and therefore already elevated at baseline. Consequently, relative to controls, IBS patients have heightened state anxiety, cortisol and HR at baseline (Heitkemper et al., 1996; Patacchioli et al., 2001) and in response to an acute psychological stressor (Dinan et al., 2006). Critically, these exaggerated elements of the stress response in IBS patients worsen their gastrointestinal (GI) symptom severity in the acute phase following a stressor (Dickhaus et al., 2003; Kennedy et al., 2014; Murray et al., 2004).

The gold-standard method for assessing acute stress reactivity and recovery in the lab is the Trier Social Stress Test (TSST) (Kirschbaum et al., 1993). The TSST involves three stress-inducing tasks: an impromptu speech preparation period, a speech task, and a mental math task. The participant performs each task in front of a panel of stone-faced judges. Unbeknownst to the participant, the judges are in on the experiment; nonetheless, the presence of the judges adds a social evaluation component that increases the stressfulness of the task. Kennedy and colleagues (2014) used the TSST and found that women with IBS had an elevated stress response and slower recovery compared to healthy controls. The exaggerated stress response for IBS patients was also associated with worsened GI symptom severity; however, the effect of stress on GI symptoms was delayed and did not peak until about one hour after the stress test. Indeed, it is in this acute recovery phase following a stressor that IBS patients often report the greatest psychological stress and physical gut discomfort (Dickhaus et al., 2003; Murray et al., 2004; Posserud, 2004).

Stress-induced gut discomfort in IBS is caused by the release of pro-inflammatory cytokines that inflame the gut (Dinan et al., 2006). Proinflammatory cytokines, such as

interleukin-6 (IL-6) upregulate indoleamine-2,3-dioxygenase (IDO) activity, which is a key enzyme in the kynurenine pathway that is responsible for tryptophan anabolism. Increasing IDO activity disrupts GI motility, increases GI sensitivity, and reduces microbiota diversity (Clarke et al., 2009; Kaszaki et al., 2008; Slattery et al., 2006). An inflamed gut also negatively impacts brain health and increases the risk of psychological disorders such as depression and anxiety (Costello et al., 2019; Gialluisi et al., 2020; Howren et al., 2009).

Inflammation in the gut is communicated to the brain via the vagus nerve, which physically connects the two. The vagus nerve responds to any deviations from homeostasis through autonomic regulation of breathing, HR, blood pressure, circulation, digestion, and gut motility. It does so by modulating the relative activation of the sympathetic (SNS; 'fight or flight') versus parasympathetic (PNS; 'rest and digest') nervous systems, which is reflected in the beat-to-beat variability of the heart called heart rate variability (HRV).

Given the role of the vagus nerve in SAM axis deactivation, measuring vagal activity via HRV provides critical insight to the stress response in both healthy and IBS patients. HRV is commonly depicted in the frequency domain using power spectra. Low frequencies (LF), 0.04 Hz to 0.15 Hz, reflect the activity of both the SNS and PNS, whereas high frequencies (HF), 0.25 Hz to 0.30 Hz, mostly reflect the activity of the PNS (Shaffer & Ginsberg, 2017). Therefore, the LF/HF ratio provides an index of the equilibrium between SNS and PNS activation, called sympathovagal balance, whereby a higher LF/HF ratio reflects greater SNS dominance and is indicative of greater stress activity (Shaffer & Ginsberg, 2017; Yilmaz et al., 2018). Relative to healthy controls, the LF/HF ratio is elevated in IBS patients at baseline (Salvioli et al., 2015; Liu et al., 2013) and this is directly related to higher levels of proinflammatory cytokine IL-6 (Stein et al., 2008; Williams et al., 2019). Critically, the LF/HF ratio is also sensitive to acute stress

elicited by the TSST; however, this has only been examined in healthy participants (Sghir et al., 2012). It follows that the LF/HF ratio may provide a marker for stress-induced inflammation that impacts the gut and brain of IBS patients.

The Benefits of Physical Activity for IBS Patients

If the LF/HF ratio is sensitive to the stress-induced inflammation that impacts the gut and brain of IBS patients, then it may be modulated by the anti-inflammatory effects of regular physical activity (Hajizadeh Maleki et al., 2018; Zheng et al., 2019). A program of regular physical activity that reduces inflammation also improves mood and reduces symptoms of depression and anxiety (Paolucci et al., 2018; Schuch et al., 2018; Werneck et al., 2020). By lowering inflammation, physical activity increases microbiota diversity (Pedersini et al., 2020; Shahar et al., 2020) and downregulates IDO activity to reduce the number of metabolites produced through the kynurenine pathway (Anderson & Maes, 2013; Nicholson et al., 2021). This counteracts the deleterious upregulation of IDO activity that is typically observed in IBS patients and helps to decrease GI sensitivity and excessive intestinal motility (Clarke et al., 2009; Kaszaki et al., 2008; Slattery et al., 2006). Furthermore, a program of regular physical activity also reduces the LF/HF ratio (Melanson & Freedson, 2001; Pearson & Smart, 2018; Picard et al., 2021) and speeds recovery from an acute stressor (Mücke et al., 2018).

The benefits of physical activity for IBS were demonstrated by one study that showed three months of light-to-moderate intensity aerobic physical activity reduced psychological symptoms, lowered pro-inflammatory cytokines and reduced abdominal distention in IBS patients compared to non-active controls (Hajizadeh Maleki et al., 2018). Another study tracked IBS patients' activity levels during a 12-week intervention and followed up with them five years later (Johannesson et al., 2015). During the 12-week intervention, the IBS patients who increased

their light-to-moderate physical activity from three hours per week to five hours per week experienced a reduction in their IBS symptom severity (Johannesson et al., 2011). Five years later, the IBS patients who were still physically active had fewer GI symptoms and were less depressed and anxious (Johannesson et al., 2015). Interestingly, these improvements in IBS symptom severity from regular physical activity were not related to cardiorespiratory fitness, perhaps because most IBS patients reported engaging in light activities (walking, cycling and aerobic classes), which are less likely to increase cardiorespiratory fitness yet still reduce symptoms comparably to vigorous exercises (Fani et al., 2019; Johannesson et al., 2018; Zhou et al., 2019). Critically, however, it remains unclear whether physical activity reduces the impact of an acute stressor on the stress reactivity and recovery of IBS patients compared to healthy controls, and this was the objective of the present study.

Aim: We aimed to investigate the effects of acute psychological stress on state anxiety, the LF/HF ratio and GI symptom severity in IBS patients and to healthy controls. Due to the COVID-19 pandemic, the psychological stress implemented was the TSST modified for remote administration. Three prior studies have implemented electronic versions of the traditional TSST (i.e., e-TSST) and found it to be effective at inducing a stress response (Eagle et al., 2021; Hawn et al., 2015; Rabasco & Sheets, 2016).

Hypotheses: Based on prior research (Dickhaus et al., 2003; Kennedy et al., 2014; Murray et al., 2004; Posserud, 2004), we expected GI symptoms to worsen during the acute phase following the stressor, and that symptoms would be worse for participants with higher baseline stress and those who experienced greater stress in response to the e-TSST. In contrast, given the protective effects of physical activity for IBS, we expected those who were more physically active to have less severe GI symptoms in the acute phase following the stressor.

METHODS

Participants

The sample size for this study was estimated using a medium effect size ($\eta^2 = 0.075$) reported by Kennedy and colleagues (2014) who also examined differences in stress reactivity and recovery between IBS patients and healthy controls after exposure to an in-person version of the TSST. Based on *a priori* calculation (G*Power version 3.1.9.3; Faul et al., 2009), we planned to recruit 26 participants, 13 IBS patients and 13 healthy controls, with an alpha (α) of 0.05 and power of 0.80. However, due to limited enrollment of IBS patients, post-hoc power analysis of 0.82 was achieved with 22 participants (9 IBS patients and 13 healthy controls) and therefore, data collection was stopped.

Participants were recruited from McMaster University using the undergraduate Psychology Neuroscience and Behaviour Research Participation System (SONA) and the Okanagan Charter. Broader recruitment also took place across Canada using social media advertising on Instagram and Facebook platforms. Finally, the Farncombe Digestive Disease Research Institute's clinical research webpage was used for targeted recruitment of IBS patients.

Eligible participants included healthy females and those with IBS aged 18-50 years old since this disorder affects women more commonly than men (Mulak et al., 2014) and given that IBS symptom severity decreases with age (Palsson et al., 2003). Due to the virtual nature of the study, participants were required to have access to a computer, webcam, and smartphone (iOS 9.0 or later, Android 4.3 or later) for the duration of the two-hour visit. IBS patients were required to meet Rome IV criteria for diagnosis which includes recurrent abdominal pain for three days of the month within the last three months and two of the following: pain or discomfort improves with defecation, pain or discomfort is associated with a change in stool frequency,

and/or pain or discomfort is associated with a change in stool appearance. Both healthy and IBS participants were excluded if they were vegetarian or vegan, had a chronic disease(s) (including diabetes, other metabolic disorders, cardiac disease, infectious disease, or cancer), had used antibiotics within the past two weeks, or were obese (Body mass index ≥ 30). Finally, although not excluded during recruitment, participants were removed from the dataset afterward if they had previously participated in the TSST (n = 1). This study received clearance from the Hamilton Integrated Research Ethics Board (#11551). During the visit, participants received a free smartphone application and following study completion, participants were compensated for their participation.

Materials

Psychological Measures

State anxiety. Psychological stress before and during the e-TSST was measured using the State-Trait Anxiety Inventory (Short Form: STAI-6; Marteau & Bekker, 1992). The STAI-6 is sensitive to acute changes in state anxiety and comparable to the full form (Marteau & Bekker, 1992). The 6-item inventory assesses state anxiety as respondents indicate how calm, tense, upset, relaxed, content and worried they feel at that moment (1 = not at all, 2 = somewhat, 3 = moderately, and 4 = very much). The STAI-6 is scored by reversing the three positive items and summing all items for a total score. The maximum score is 24, where higher scores reflect higher levels of state anxiety.

Depression. Depression was measured using the Beck Depression Inventory-II (BDI-II) questionnaire (Beck et al., 1988), which is a valid tool for assessing depressive symptoms in both clinically depressed patients and healthy populations (Beck et al., 1988). The BDI-II is a 21-item tool that assesses depression symptom severity over the past two weeks through ratings on a

scale from 0 to 3, where lower ratings reflect fewer depressive symptoms. Each item is summed for a total score. Higher scores reflect more severe depression symptoms. Depression was included as a covariate when assessing the relationship between physical activity and GI symptom severity given that increased depressive symptoms, such as catastrophizing, are associated with greater pain severity in IBS (Lackner et al., 2004).

Chronic stress. Chronic stress was measured using Cohen's Perceived Stress Scale (PSS) (Cohen et al., 1983). Research in adult populations shows the tool is valid and sensitive to chronic stress levels (Roberti et al., 2006). The 10-item questionnaire had respondents indicate how frequently they experienced stress symptoms over the past month using a rating scale (0 = never, 1= almost never, 2 = sometimes, 3 = often, and 4 = very often). The questionnaire is scored by reversing the four positively stated items and adding each item. The maximum score is 40 where higher scores reflect higher levels of perceived stress. Chronic stress was also included as a covariate when assessing the relationship between physical activity and GI symptom severity given the strong association between IBS symptom severity, anxiety, and the presence of chronic stressors in one's life (Gulewitsch et al., 2013).

Physiological Measures

The LF/HF ratio. The LF/HF ratio is a valid and reliable measure of physiological stress reactivity (Kim et al., 2018). The LF/HF ratio was measured using photoplethysmography (PPG) through the smartphone application, Camera HRV, which has been validated by research and yields comparable results to the gold-standard measurement of an electrocardiogram (ECG) (Plews et al., 2017). The application instructs the user to place their index finger over their camera and uses both the flashlight (torch) and camera to measure changes in light absorption and detect changes in blood volume during a heartbeat. A single measure takes one minute to

compute. During the measure, participants were instructed to follow a breathing bar set at a default rate of ten breaths per minute. Every second during the measurement minute, the application recorded HR and at the end of the minute, it reported the LF/HF ratio, which was used as an index of sympathovagal balance.

GI Symptom Severity. To measure IBS symptoms and stress-evoked GI disturbances, Part 1 (Severity Score) of the IBS Symptom Severity Score (IBS-SSS) questionnaire was used (Francis et al., 1997). The tool is valid for assessing IBS symptom severity (Francis et al., 1997) and is sensitive to acute fluctuations in gut discomfort (Kennedy et al., 2014). It assesses GI symptom severity using 4-items that have participants self-report acute abdominal pain, abdominal distension, the urge to have a bowel movement, and perceived impairment on quality of life. Each item is rated using a visual analog scale and summed for a total score where higher ratings reflect greater GI symptom severity and discomfort. The tool proposes scores ≥ 15 are indicative of a clinical diagnosis of IBS with classifications for mild (16-34), moderate (35-59) and severe (> 60) IBS symptoms (Francis et al., 1997).

Lifestyle Measures

Physical activity. Physical activity levels were assessed using the Stanford Seven-Day Physical Activity Recall (7DPAR) questionnaire. The 7DPAR questionnaire is valid for interview-administered recall and is consistent with measures of cardiorespiratory fitness (Dishman & Steinhardt, 1988; Schilling et al., 2018). Furthermore, the tool is valid in special populations as it identifies patients at both the high and low extremes of physical activity levels (Garfield et al., 2012). The 7DPAR determines weekly energy expenditure (kcal/kg/week) by asking participants to recall the moderate, hard, and very hard intensity physical activity they engaged in over the past seven days. To increase recall reliability, participants indicate the time

of day (morning, afternoon, evening) and duration (hours). Weekly durations of physical activity at the various intensities are multiplied by a constant metabolic equivalent of the task (MET) to determine energy expenditure. Higher energy expenditures reflect greater physical activity with suggested classifications of low (< 245 kcal/kg/week) and high active (> 280 kcal/kg/week) from Dishman & Steinhardt (1988).

Sleep. The Pittsburgh Sleep Quality Index (PSQI) questionnaire (Buysse et al., 1989) was used to determine sleep quality. This lifestyle measure was used as a covariate in analyses with physical activity and GI symptom severity given the strong positive correlation between physical activity and sleep (Bellini et al., 2011) and negative correlation between sleep and GI symptom severity (Patel et al., 2016). The PSQI is a valid tool for measuring sleep quality in both healthy and IBS patients, as it has shown to be sensitive to differences in sleep dysfunction in clinical and non-clinical populations (Mollayeva et al., 2016). The questionnaire assesses overall sleep quality over the last month by looking at subjective sleep quality, sleep latency, sleep duration, sleep efficiency, difficulties sleeping, use of sleeping medications, and day-time sleepiness. Each item is scored by the severity or frequency of sleep disturbances (where 0 = least severe or frequent, 1 = fairly severe or frequent, 2 = quite severe or frequent, and 3 = most severe or frequent) and summed to get a total score. The maximum score is 21, where higher scores are indicative of greater sleep disturbance, and a score of > 5 indicates a poor sleeper (Buysse et al., 1989).

Medication Use. During the screening assessment, participants were asked whether they were taking any prescribed medication, over the counter (OTC) products, or supplements. Any medications that could have affected the autonomic nervous system (ANS), including antidepressants, beta-blockers, calcium-channel blockers, and benzodiazepines were indexed. To

control for any confounding effects in analyses with state anxiety and the LF/HF ratio, participants that reported taking medications affecting the ANS were coded as "1", while those that did not report the use of these medications were coded as "0".

Procedure

Screening Protocol

Interested participants completed a 30-minute phone screening prior to study enrollment. During the phone screening, measures of physical activity and current medication use were collected.

Virtual Visit

After completing screening, participants were invited to participate in a virtual study visit through the video conferencing platform Zoom. To begin, participants completed a series of online questionnaires using the tool *Limesurvey*. The questionnaires collected information regarding age, depression, chronic stress, and sleep.

Following survey completion, participants downloaded the Camera HRV application onto their smartphones and took a test measurement. After successful completion of a test measure, baseline measures of state anxiety, the LF/HF ratio and GI symptom severity were collected. Participants were then exposed to a fully remote e-TSST based on the in-person test design (Kirschbaum et al., 1993). To start, two research assistants posing as judges were invited to the Zoom call and told the participant that they would need to complete a cognitive task. The judges described the e-TSST protocol to the participant and told the participant that they would be evaluating her body language and non-verbal communication.

The e-TSST comprised of three five-minute tasks. During the first five minutes, the participant prepared a speech describing herself as the ideal candidate for her dream job, and she was encouraged to write down her ideas either using a pen and paper or an electronic device (i.e., computer, iPad, or smartphone). After that, she delivered her speech to the judging panel, who were instructed to show neutral expressions throughout. Five minutes were allotted for the speech delivery task, and if the participant fell silent, she was instructed to continue talking until the time was up.

After the speech delivery, the participant completed a mental arithmetic task for five minutes. This task required her to sequentially subtract 13 from 1022 as quickly and accurately as possible. Again, this was done in front of the judges who forced the participant to restart if the incorrect answer was given and monitored the participant to ensure she did not use any writing tools or a calculator.

The virtual visit continued for one hour after the e-TSST (i.e., the recovery phase) during which time the participant sat quietly and watched an emotionally neutral Planet Earth video.

Measures of state anxiety, the LF/HF ratio and GI symptom severity were collected at six time points: (1) baseline, (2) midway through the e-TSST (immediately following the speech delivery but before the mental math task), (3) at the end of the e-TSST (denoted as t+0), and (4, 5, and 6) every 20 minutes thereafter up to one hour after completion of the e-TSST (denoted as time points t+20, t+40 and t+60).

At the end of the virtual visit, the true nature of the e-TSST was disclosed and participants were probed for previous knowledge or participation in the test.

Statistical Analysis

To examine differences between IBS patients and healthy controls, *t*-tests were computed for baseline psychological, physiological, and lifestyle measures. A chi-squared test was computed to test the difference in the proportion of anti-depressant medication use between groups. To further characterize the present sample, correlations were conducted between descriptive characteristics.

To examine psychological and physiological stress reactivity and recovery, three separate mixed linear model analyses were conducted on state anxiety, the LF/HF ratio and GI symptom severity with a between-subject factor of group (IBS, controls) and a within-subject factor of time, using the six measurements taken before, during and after the e-TSST.

To examine whether higher stress was associated with more severe GI symptoms during recovery, we computed correlations between measures of state anxiety and the LF/HF ratio with GI symptom severity at the four time points following the e-TSST.

To examine whether higher baseline stress was associated with more severe stress-induced GI disturbances during recovery, we computed correlations between *baseline* measures of state anxiety and the LF/HF ratio with GI symptom severity at the four time points following the e-TSST.

Finally, to examine whether IBS patients who were more physically active had less severe GI symptoms during recovery, we computed correlations between the 7DPAR weekly energy expenditure scores and GI symptom severity at the four time points following the e-TSST. Depression, chronic stress and sleep quality were included as covariates to assess the

complex interplay between these lifestyle factors and the protective effects of physical activity on the gut.

Data were analyzed using IBM SPSS Statistics Software 26. For all statistical analyses, a *p*-value (two-tailed) of < 0.05 was considered significant, except for the correlation analyses between physical activity and GI symptom severity which were one-tailed. One-tailed analyses were conducted between these variables given the extensive literature supporting our directional hypothesis whereby physical activity negatively correlates with GI disturbances (Fani et al., 2019; Hajizadeh Maleki et al., 2018; Johannesson et al., 2011; Zhou et al., 2019). The use of antidepressant medication, which was coded as "1", was controlled for in correlational analyses involving state anxiety and the LF/HF ratio given their effect on mood and the ANS (Yeh et al., 2016).

RESULTS

Data Screening and Assumptions

Data were screened for normality using the Kolmogorov-Smirnov test and through visual inspection of histograms. GI symptom severity was non-normal at all time points, while all other variables were normal. Thus, non-parametric analyses, Mann-Whitney U test and Spearman's correlation (r_s), were conducted with GI symptom severity. Data were screened for extreme outliers using the following criteria: values beyond quartiles 1 and 3 with a step of 3 times the interquartile range; and based on this criterion, no extreme outliers were detected.

Data were screened for missingness. High missingness for the LF/HF ratio (13.6-45.5%) was due to poor signal quality of the Camera HRV smartphone application. Data that had missingness > 10% (LF/HF at all six time points) were subsequently imputed using expectation-

maximization (EM) (Bennett, 2001; Tabachnick et al., 2019). EM imputed values that were outside the physiological range (< 0) were removed. All analyses were computed with imputed LF/HF data.

For all other data, missingness ranged from 0-9.1%. These missing data at 9.1% were for state anxiety (n = 2; 1 healthy control and 1 IBS patient) and GI symptom severity (n = 2; 1 healthy control and 1 IBS patient). These missing data were due to lost internet connection during the virtual visit. For all data, the pattern of missingness was missing completely at random (MCAR), according to Little's MCAR test.

Descriptive Characteristics

Table 1 presents descriptive characteristics comparing IBS patients with healthy controls who were between 18-42 years old; on average, IBS patients were a few years older than the controls. Both groups reported minimal depressive symptoms (BDI-II < 13) but (based on population norms) had above-average levels of chronic stress (PSS > 13) and slept poorly (PSQI > 5). All IBS patients were above the IBS diagnostic cutoff of 15 and were experiencing more severe GI symptoms at baseline than the healthy controls. State anxiety and the LF/HF ratio at baseline were also higher in IBS patients than healthy controls, suggesting that IBS patients were already in a high-stress state prior to the acute stressor. Both groups reported relatively low levels of physical activity, with the IBS patients just surpassing the low active cut-off (> 245 kcal/kg/week) and healthy controls falling below it, though these group differences were not significant. Additionally, no participants reported having COVID-19 or related symptoms.

With respect to medication use, a total of six participants reported taking prescribed medication, over-the-counter products, and supplements (IBS, n = 4; Controls, n = 2). Table 2 contains a detailed list of the products participants reported taking. Notably, more IBS patients

reported taking antidepressant medications than controls; however, this group difference was not significant (X^2 (1, N = 22) = 1.04, p = .31).

Table 3 presents the correlations between *baseline* psychological, physiological, and overall lifestyle measures. Higher chronic stress was associated with more depressive symptoms and worse quality of sleep, and higher state anxiety was associated with greater GI symptom severity at baseline.

Stress reactivity and recovery to the e-TSST

The mixed linear models for state anxiety, the LF/HF ratio and GI symptoms severity are presented in Table 4. State anxiety (Figure 1A) revealed a main effect of time but no effect of group such that both IBS patients and healthy controls experienced elevated anxiety in response to the e-TSST. The LF/HF ratio and GI symptom severity models revealed a main effect of group but no effect of time indicating that both the LF/HF ratio and GI symptom severity were elevated in IBS patients compared to controls across all time points (Figures 1B and C).

In the acute phase following the e-TSST, participants with higher LF/HF ratios had worse GI symptom severity during the first 20 minutes of recovery but not later (Table 5). However, state anxiety and GI symptom severity were not associated at any of the time points.

Higher baseline stress was also associated with more severe stress-induced GI symptoms during recovery. Specifically, baseline state anxiety was associated with greater GI symptom severity at all time points during recovery (Table 6). However, baseline LF/HF ratio was not.

IBS patients who were more physically active had less severe GI symptoms during recovery. Higher physical activity in IBS patients was associated with lower GI symptom severity at time point t+20 (r(9) = -0.72, p = 0.02; Figure 2) and all other timepoints ($p \leq 0.03$).

When these correlations between physical activity and GI symptom severity were re-run controlling for depression, chronic stress, and sleep quality, they were no longer significant. Additionally, the relationship was not significant when the data from healthy controls were including, suggesting that this is an IBS-specific association.

DISCUSSION

The present study used the e-TSST to examine the impact of an acute stressor on stress reactivity and recovery in IBS patients compared to healthy controls. State anxiety, the LF/HF ratio and GI symptom severity were elevated in IBS patients relative to healthy controls. Although only state anxiety levels changed throughout the e-TSST, GI symptoms following the e-TSST were aggravated when stress levels were higher at baseline and in response to the acute stressor and were alleviated in patients who were physically active.

All participants showed signs of poor mental health evidenced by their higher-than-expected levels of chronic stress and poor sleep, likely because of data collection took place during the global pandemic (Marashi et al., 2021); however, IBS patients suffered more. For one, their state anxiety—i.e., the momentary anxiety due to current circumstances—was elevated at baseline, suggesting a heightened anticipatory stress response. Furthermore, their heightened anxiety was associated with worse GI symptoms severity following the stressor. This suggests that a heightened anticipatory stress response may exacerbate subsequent gut symptoms brought on by a stressful experience. Indeed, activation of the stress response increases inflammation and kynurenine metabolites that worsen GI symptoms (Clarke et al., 2009; Dinan et al., 2006; Kaszaki et al., 2008; Slattery et al., 2006), which the brain interprets as a threat. This causes increased state anxiety that signals the body to keep the HPA axis “on”, thus, creating a vicious cycle through which the inflammatory and kynurenine pathways exacerbate GI symptoms and

anxiety even more. Although the relationship is bidirectional, our findings lend further support for mental health impacting gut health (Blanchard et al., 2008; Fond et al., 2014; Lackner et al., 2004, 2013).

IBS patients also had higher LF/HF ratios throughout the experiment, suggesting a constant state of "fight or flight" that is indicative of chronic stress. This enhanced vagal activity reflects the constant basal activation of the SAM axis in IBS patients, linking decreased parasympathetic activation to increases in circulating catecholamines in this population (Heitkemper et al., 1996). Furthermore, patients with a higher LF/HF ratio had worse GI symptoms during the first *20 minutes* following the acute stressor. This finding is like prior work by Kennedy and colleagues (2014) that demonstrated delayed gut discomfort in IBS patients *60 minutes* after the TSST; however, note the time difference between their study and ours. One explanation for the time difference is that Kennedy et al. (2014) only assessed GI symptom severity at two time points: immediately following the TSST (t+0) and 60 minutes later (t+60). If they had taken more measures, then they may have observed a similar peak as we did at the 20-minute mark. That said, the time difference in peak gut discomfort may be related to the absolute differences in IBS severity between the studies. In our study, IBS patients' symptoms were mildly intense (IBS-SSS: 16-34) and increased to moderately intense at 20 minutes following the stressor. Whereas in Kennedy and colleagues' study (2014), the IBS patients' symptoms were moderately intense (IBS-SSS: 35-59) and increased to severely intense (IBS-SSS: > 60) at 60 minutes following the stressor. Therefore, it is possible that the severity of GI symptoms also played a role in increasing the time to peak following the stressor. Further research is needed to investigate this.

Importantly, physical activity was protective against gut disturbances in IBS patients during the acute phase following the stressor. Specifically, IBS patients who were more physically active had less severe GI symptoms through the acute phase following a stressor. This protection from physical activity may be due to the anti-inflammatory effects, and the downstream effects on the kynurenine pathway, gut motility and GI sensitivity. It may also be related to up-regulation of GR expression (Pan-Vazquez et al., 2015), which may explain why the protective effects of physical activity on GI symptom severity especially during the recovery period post-stressor. It would follow that increased expression of GRs in physically active IBS patients may increase the efficiency of cortisol's negative feedback loop, allowing the HPA axis to turn "off" once a stressor has passed and thus reducing downstream GI disturbances.

How much physical activity is needed to reap these benefits? Visual examination of Figure 2 suggests the association between more physical activity and less GI symptoms plateaus beyond the criteria for "low" physical activity (i.e., 7DPAR $\leq$ 245 kcal/kg/week) suggesting a threshold effect. This observation is consistent with prior work that suggests IBS patients require low-to-moderate levels of physical activity to reap the benefits of attenuated GI symptom severity (Johannesson et al., 2015). Therefore, low levels of weekly physical activity may be an effective strategy for IBS symptom management without any adverse GI reactions. This messaging is in line with prior studies that have found that an acute bout of vigorous physical activity can worsen GI symptom severity and discomfort by increasing GI permeability and endotoxemia while also slowing gastric emptying and intestinal transit (Costa et al., 2017). This information is critical for informing physical activity guidelines for IBS symptom management.

Interestingly, the protective effects of physical activity on GI symptoms were no longer significant after controlling for depression, chronic stress, and sleep quality. This suggests that

the benefits of physical activity for IBS may be related to the broader benefits of physical activity for mental health. Physical activity is associated with a better mood, less stress and higher quality sleep (Ghrouz et al., 2019; Rosenbaum et al., 2014; Wang & Boros, 2021; Wunsch et al., 2017), which would all help interrupt the vicious cycle between higher anxiety and worse GI symptoms that tends to play out in IBS patients. It follows that other lifestyle factors known to improve mental health, such as mindfulness, may also help ease the stress-induced GI symptoms in IBS patients. Mindfulness and breath awareness have been shown to reduce the LF/HF ratio and GI symptom severity in response to a stressor (Takahashi et al., 2005; Wu & Lo, 2008; Zernicke et al., 2013). Therefore, a combined approach of increasing physical activity and mindfulness may be optimal for IBS management and should be the focus of future studies.

Surprisingly, although IBS patients had higher stress activity at baseline, their reactivity to the e-TSST was not significantly different from healthy controls as we had expected. However, it is important to note that this study was conducted within the first year of the COVID-19 pandemic and reactivity by both groups was likely marred by the chronic stress caused by this global health crisis. Evidence for this is supported by higher than expected chronic stress levels in healthy controls seen in our sample, which aligns with prior reports taken during the same timeframe (Marashi et al., 2021). Therefore, the chronic stress and anxiety caused by the ongoing external circumstances may have overshadowed any potential group differences in anxiety-evoked by the e-TSST.

In addition to the important theoretical contributions above, this study provides important methodological contributions that help inform methods for remote testing. As the first study to use the e-TSST in IBS patients, we observed an increase in state anxiety in both IBS patients and healthy controls, confirming that the e-TSST can induce a stress response. Our results add to the

three prior studies that implemented modified internet versions of the TSST in healthy participants (Eagle et al., 2021; Hawn et al., 2015; Rabasco & Sheets, 2016). Of the three prior studies, Eagle and colleagues' study (2021) was most similar to ours because they are the only ones who also used Zoom to administer the e-TSST and measured changes in perceived stress and HRV. However, there are key methodological differences between the two studies. Notably, Eagle and colleagues (2021) used a *single* Zoom account to administer the e-TSST meaning that the research assistant and judges were all at the same physical location and appeared on-screen at different times. Contrastingly, in the current study, the research assistant and TSST judges (n = 2) used *separate* Zoom accounts and joined the videoconference call from different physical locations. Our version gave participants a better view of the judge's facial expressions, which likely explains the larger effect size was observed here ($\eta_p^2 = 0.54$) as compared to the medium effect size reported by Eagle and colleagues ($\eta_p^2 = 0.17$). Therefore, we recommend the use of multiple Zoom accounts when using the e-TSST.

Another difference between the two studies concerns the measurement of HRV. Eagle and colleagues (2021) mailed each participant an ECG monitor to collect HRV, which is the gold standard method for HRV data acquisition (Khunti, 2014). Resultingly, they were able to detect HR differences in response to the e-TSST that were not observed in our study. In contrast, our acquisition of HRV as index by the LF/HF ratio used a smartphone application, which uses PPG technology. Although this methodology has been validated in the literature (Plews et al., 2017), it was subject to poor signal quality and extensive missing data. Therefore, we recommend the use of ECG for measures of HRV in future remote studies.

CONCLUSION

In summary, IBS patients had higher stress levels and more severe GI symptoms than healthy controls. In the acute phase following a stressor, GI symptoms were more severe in participants with higher stress but were alleviated in IBS patients who were physically active. Given the importance of IBS symptom management on quality of life, these results point to the promise of physical activity as a strategy to help patients to manage their GI symptoms in response to acute stressors.

FIGURES

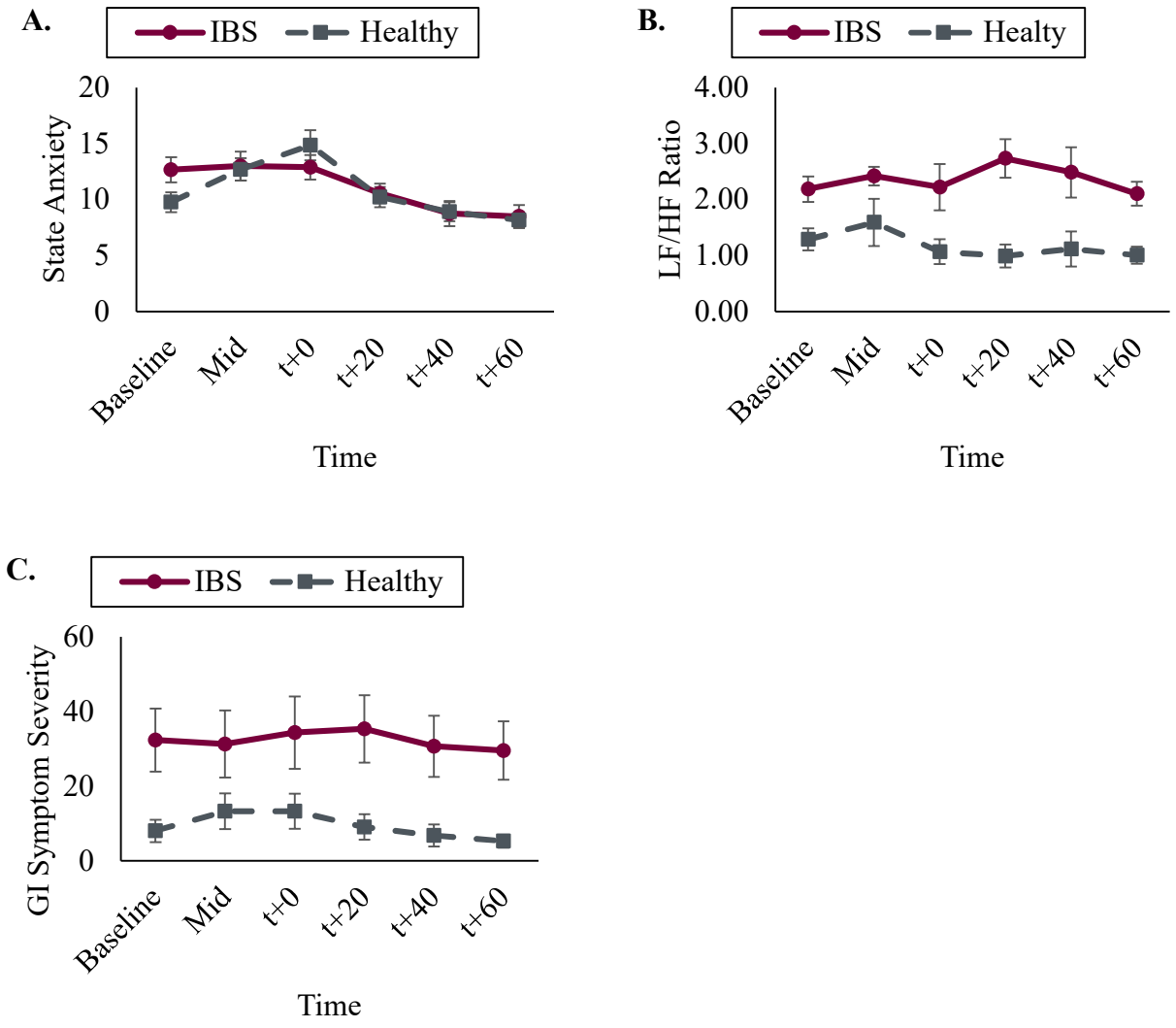

Figure 1. State anxiety, the LF/HF ratio and GI symptom severity before during and after the e-TSST. **A)** The e-TSST had a significant effect of time ($F(5, 115) = 7.70$ $p < .001$) but no effect of group ($F(1, 115) = 0.52$ $p = .47$) on state anxiety. **B and C)** The e-TSST had a significant effect of group on the LF/HF ratio ($F(1, 116) = 24.95$ $p < .001$) and GI symptom severity ($F(1, 115) = 71.54$ $p < .001$). Analyses with state anxiety and the LF/HF ratio include antidepressant medication use as covariates.

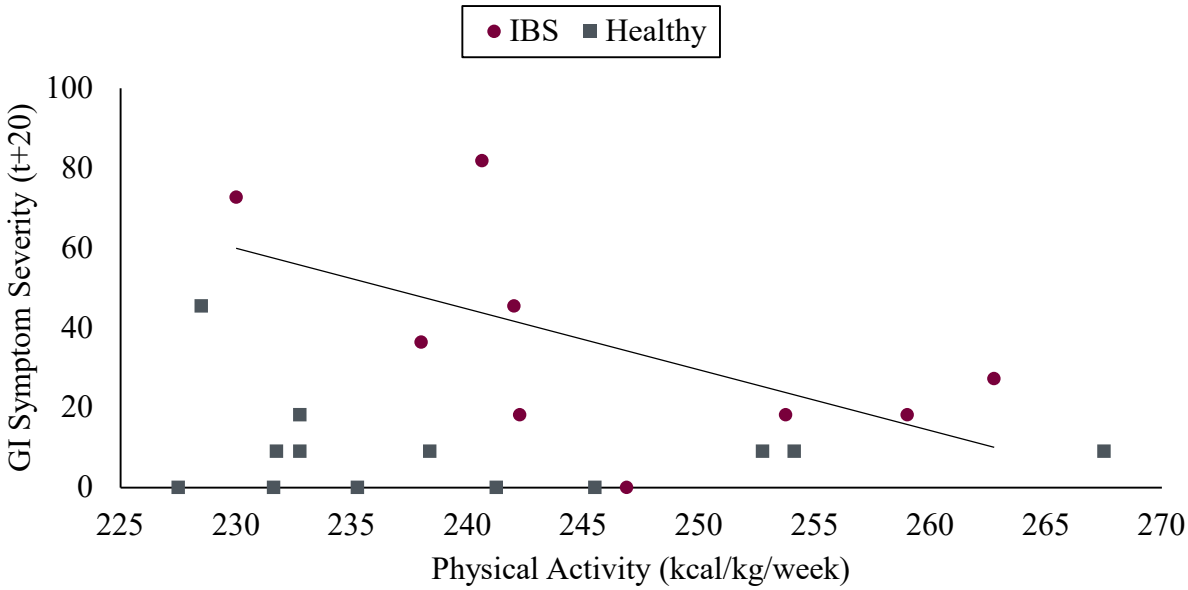

Figure 2. Higher physical activity was associated with less severe GI symptoms 20 minutes after the e-TSST. A negative correlation was observed between physical activity and GI symptom severity at t+20 in IBS patients ($r_s(9) = -0.66$, $p = .03$). Note that a similar negative correlation was observed between physical activity and GI symptom severity in IBS at all time points post stressor.

TABLES

Table 1. Descriptive characteristics of participants at baseline and overall lifestyle measures.

	IBS Patients (n = 9)	Healthy Controls (n = 13)	*p*-value
Age	29.67 ± 6.67	22.23 ± 7.42	0.03*
State Anxiety	12.67 ± 3.39	9.77 ± 3.22	**.05***
Depression	11.11 ± 7.69	11.08 ± 7.59	.99
Chronic Stress	19.89 ± 8.68	18.85 ± 5.27	.72
LF/HF Ratio	2.19 ± 0.60	1.29 ± 0.69	**.01***
GI Symptom Severity	32.32 ± 25.35	8.04 ± 10.84	**.01***
Physical Activity (kcal/kg/week)	246.14 ± 10.54	239.97 ± 11.93	.23
Sleep Quality	5.67 ± 1.87	5.92 ± 1.98	.76

Data given as mean ± standard deviation (S.D.), *$p \leq .05$.

Table 2. Participants' medications, OTC products and supplements.

Drug Class	Drug/Brand Name	IBS Patients	Healthy Controls
Antidepressants	Clonazepam, Duloxetine, Escitalopram, Paroxetine, Sertraline, and Venlafaxine	4	2
OTC Products, Supplements and Vitamins	Gaviscon, Iron Bisglycinate, Nasal Spray, Omega-3, Probiotics, Vitamin B12, Vitamin C and Vitamin D	3	4
Acne Medication	Isotretinoin	0	1
Contraceptive	Alesse, Evra and Lolo	3	0
Asthma Medication	Budesonide/formoterol	1	0
IBS Medication	Pinaverium and Prucalopride	2	0
Anticholinergic Medication	Glycopyrrolate	1	0

Table 3. Correlations between baseline psychological, physiological and lifestyle measures.

	1.	2.	3.	4.	5.	6.	7.
1. State Anxiety	-						
2. Depression	0.34	-					
3. Chronic Stress	0.24	**0.67****	-				
4. LF/HF Ratio	0.43	0.24	0.45	-			
5. GI Symptom Severity	**0.47***	0.20	0.09	0.41	-		
6. Physical Activity	-0.18	**-0.40**†	-0.02	-0.06	0.07	-	
7. Sleep Quality	0.07	0.33	**0.59****	0.40	-0.20	0.05	-

Note: Analyses with GI symptom severity report Spearman's correlation coefficients, while all others report Pearson's correlation coefficients. Correlations involving state anxiety and LF/HF ratio control for anti-depressant medication use. Two-tailed †$p = .07$ (trending), *$p < .05$, **$p < .01$.

Table 4. Mixed linear model analyses examining group differences between IBS and healthy controls at six time points before, during and after the e-TSST for state anxiety, the LF/HF ratio and GI symptom severity.

	Group	*Time*
State Anxiety	0.52	**7.70****
LF/HF Ratio	**24.95****	1.67
GI Symptom Severity	**71.54****	0.27

Note: Values are *F* statistic. **$p \leq .01$.

Table 5. Correlations between acute stress and GI symptom severity following the e-TSST.

	GI Symptom Severity			
	t+0	*t+20*	*t+40*	*t+60*
State anxiety	0.35	0.28	0.01	0.21
LF/HF Ratio	**0.49***	**0.75****	0.05	0.51

Note: Values are Spearman's correlation coefficients and control for anti-depressant medication use. Two-tailed *$p < .05$, **$p < .01$.

Table 6. Correlations between baseline levels of stress and physical activity with GI symptom severity following the e-TSST.

	GI Symptom Severity			
	t+0	*t+20*	*t+40*	*t+60*
Baseline State Anxiety	**0.51***	**0.51***	**0.47***	**0.48***
Baseline LF/HF Ratio	-0.00	0.09	0.12	0.16

Note: Values are Spearman's correlation coefficients. Analyses with state anxiety and the LF/HF ratio control for anti-depressant medication use and are two-tailed. *$p < .05$.

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