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Stress and Gene Expression of Individuals With Chronic Abdominal Pain

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Abstract

Background—Research examining the role of stress in gastrointestinal (GI) symptoms such as chronic abdominal pain (CAP) is controversial. The purpose of this study was to examine the expression of genes involved in metabolic stress and toxicity in men and women with high and low levels of perceived stress with and without CAP.

Methods—Data and samples were collected and the expression of genes involved in metabolic stress and toxicity was analyzed in 26 individuals who had consented to participate in a natural history protocol. Subjects completed the 10-item Perceived Stress scale (PSS). Fasting participants' peripheral whole blood was collected for proteomic and genomic studies. Polymerase chain reaction (PCR) array was used to analyze the expression of 84 key genes involved in human stress and toxicity plus 5 housekeeping genes. Plasma interleukin-1 alpha (IL-1α) protein was quantified via enzyme-linked immunosorbent assay (ELISA).

Results—Interleukin-1 alpha gene (*IL1A*) was upregulated in females with high stress versus females with low stress by 2.58-fold (95% CI [0.88, 4.28]). *IL1A* was upregulated in participants with high stress and CAP versus those with low stress and CAP by 3.47-fold (95% CI [1.14, 5.80]).

Conclusions—An upregulation of the gene coding the pro-inflammatory cytokine IL-1α suggests that the mechanism behind stress-related changes in GI symptoms is pro-inflammatory in nature. The results of this study contribute to the knowledge of the mechanism behind stress-related CAP symptoms and gender differences associated with these disorders.

Keywords

stress; abdominal pain; gene expression; *IL1A*; IL-1α

The term *stress* refers to the alterations, both positive and negative, in an organism's environment, which elicit a response from that organism. The function of the stress response

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Declaration of Conflicting Interests

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is to maintain homeostasis. As a normal function or response, the stress response is vital in supporting the body as it encounters negative changes in the environment. For the purposes of this article, we use the term *stress* to refer to both the perceived negative changes in a person's environment and the psychological, physiologic, and behavioral changes that typically result (Mawdsley & Rampton, 2005).

Research has revealed numerous negative health effects associated with stress. For example, stress causes an increased activation of inflammatory cytokines in arthritis (Edwards et al., 2009; Hirano, Nagashima, Ogawa, & Yoshino, 2001; Motivala, Khanna, FitzGerald, & Irwin, 2008) and delays in oral mucosal wound healing in healthy college students (Marucha, Kiecolt-Glaser, & Favagehi, 1998) and in skin barrier function recovery in healthy women (Altemus, Rao, Dhabhar, Ding, & Granstein, 2001). Focusing on its effects on the gastrointestinal (GI) tract, investigators have associated stress with alterations in tight junctions and barrier function (Mazzon, Sturniolo, Puzzolo, Frisina, & Fries, 2002; Rhodes & Karnovsky, 1971) and worsening of symptoms in irritable bowel syndrome (IBS; Drossman et al., 2000) and inflammatory bowel disease (IBD; Duffy et al., 1991).

Brain-gut interactions play a vital role in maintaining GI health, and disruption of these interactions is associated with symptoms in a number of disorders and syndromes, including IBS and IBD (Mayer & Tillisch, 2011). Stress negatively impacts the brain-gut axis, thus contributing to GI dysfunction (Konturek, Brzozowski, & Konturek, 2011). The specific mechanisms by which stress induces such disruption are unknown, but the effects of stress include visceral hypersensitivity; changes in motility, gut microbiota, and release of hormones and neurotransmitters; and increased expression of proinflammatory cytokines.

Inflammation is the process by which pathogens in the body are deactivated, destroyed, and or rendered incapable of infecting or affecting the body. A pathogen or physical injury damages tissue, and macrophages, after activation, release pro-inflammatory cytokines like interleukin-1 alpha (IL-1 α) that then recruit leukocytes and neutrophils to the damaged or injured area. IL-1 α , specifically, is produced by activated macrophages, neutrophils, and epithelial cells and functions primarily to increase the systemic inflammatory response by increasing systemic neutrophil counts. IL-1 α is present locally at mucosal surfaces, like skin and the GI tract, and has the potential to transform a local mucosal insult into systemic inflammation (Hauser, Saurat, Schmitt, Jaunin, & Dayer, 1986). This cytokine functions in many inflammatory pathologies such as arthritis, renal disease, graft-versus-host disease, osteoporosis, and diabetes (Arend, 2002).

As suggested above, although inflammation is a normal immune response, alterations to its mechanisms may have negative physiologic consequences. The literature, in fact, suggests an inflammatory component to stress. In the GI tract, specifically, stress is associated with disruption of the intestinal barrier to macromolecular substances and may involve stress-related cholinergic mechanisms (Kiliaan et al., 1998) related to altered motility. Corticotropin-releasing hormone (CRH), a highly studied endogenous substance related to stress and the resulting effects on the GI tract, has been associated with stress-related increases in distal colonic secretion and permeability (Santos et al., 1999). CRH is also responsible for stress-related changes in rat and human GI secretion, motility, and function (Lenz, 1990; Tache & Perdue, 2004). Additionally, cortisol and the adrenocorticotrophic hormone (ACTH)-to-cortisol ratio are commonly used physiological measures of stress (Deechakawan, Cain, Jarrett, Burr, & Heitkemper, 2011; Kuroki et al., 2010; Voss et al., 2011; Vythilingam et al., 2009).

A subclinical inflammatory component mediated by stress may be part of the molecular mechanisms in chronic abdominal pain (CAP; Barbara, 2006; R. Spiller & Garsed, 2009), a

widely acknowledged symptom that affects an estimated 15–20% of people worldwide (Creed et al., 2001; Russo, Gaynes, & Drossman, 1999). CAP is one of the main symptoms associated with functional GI disorders (Drossman et al., 2006) such as IBS, which cost the United States more than \$30 billion annually in direct medical care costs and time lost from work (Ashburn & Gupta, 2006; Spinelli, 2007; Thompson et al., 1999). Investigators have found low-grade inflammation and mast cells in mucosal biopsies of IBS and CAP patients (Barbara, 2006; Barbara, De Giorgio, Stanghellini, Cremon, & Corinaldesi, 2002; De Giorgio & Barbara, 2008; Mertz, 2003; O'Sullivan, Mahmud, Kelleher, Lovett, & O'Morain, 2000; R. C. Spiller, 2004; Taylor, Youssef, Shankar, Kleiner, & Henderson, 2010), supporting the hypothesis of an inflammatory component in this pathology.

Females report symptoms consistent with IBS more frequently than males (2:1) in the United States. This variation in reporting could reflect an increased prevalence in females or an underreporting in males, but literature does reveal that females request care for GI symptom distress more often than males (Frissora & Koch, 2005). Gender effects on CAP and IBS are considered a multifactorial phenomenon that includes inherent biological sex differences in hormones, stress and inflammatory reactions as well as sociocultural variations in pain responses and/or changes in bowel patterns (Cain et al., 2009; Frissora & Koch, 2005; M. Heitkemper & M. Jarrett, 2008; M. M. Heitkemper & M. E. Jarrett, 2008).

The purpose of this study was to examine the expression of genes involved in stress and toxicity in men and women with high and low levels of perceived stress, with and without CAP.

Materials and Methods

Design and Setting

This cross-sectional secondary data analysis included a subset of participants we recruited under a natural history protocol (09-NR-0064, [Clinicaltrials.gov](https://clinicaltrials.gov/ct2/show/study/NCT00824941) #NCT00824941) conducted at the National Institutes of Health (NIH) Hatfield Clinical Research Center. We collected biological samples and administered questionnaires over two outpatient visits from January 2009 to November 2010. The two outpatient visits were typically conducted 24 or 48 hr apart. Participants gave written consent, and the Institutional Review Board and the Office of Human Subjects Research at the NIH approved the study.

Assessment of Perceived Stress

Participants completed the Perceived Stress Scale (PSS), a subjective measure of perceived stress, on the first visit. Researchers have found the PSS to be valid and reliable for assessment of self-reported stress in a variety of disorders (Cohen, Kamarck, & Mermelstein, 1983). Scores on the PSS range from 0 to 40, but there is no clearly defined designation between high and low stress. We therefore categorized stress based on the overall distribution of the parent study, explained below, defining high stress as 1 standard deviation (*SD*) above the overall mean PSS score of the parent study and low stress as 1 *SD* below.

Sample

The subset of the parent study ($N = 56$) that we included in the present study comprised 26 participants (50% male, 58% Caucasian, mean age 26.50 ± 7.02 years) with high stress ($n = 10$) or low stress ($n = 16$) as indicated by PSS scores. Of the 26 participants, 38.5% had CAP of unknown etiology ($n = 10$). Female participants completed the protocol between Days 3 and 10 of the menstrual cycle (self-reported) to control for hormonal variation. Inclusion criteria of the parent study include participants with a history of CAP for at least the past 6

months and healthy controls. Exclusion criteria include any known (self-reported) organic disease (GI, pulmonary, neurologic, renal, endocrine, or gynecological pathology). We measured blood cortisol and ACTH levels for all participants on their first visit.

RNA Extraction

We collected fasting participants' peripheral whole blood (2.5 ml) during the second visit by venipuncture into PAXgene® RNA tubes (Qiagen, Franklin Lakes, NJ). Samples were stored at -80°C for a period of approximately 2 years prior to RNA extraction. We used the PAXgene® Blood miRNA Kit to extract RNA from blood and, as a quality control measure, we verified random RNA sample quality with the Experion™ RNA Analysis Kit (Bio-Rad, Hercules, CA).

Gene Expression

We reverse transcribed RNA (300 ng) using the RT² First Strand Kit (SA Biosciences by Qiagen, Frederick, MD). We used RT² Profiler™ PCR Array Human Stress and Toxicity (PAHS-003, SA Biosciences by Qiagen, Frederick, MD) to analyze the expression of 84 key genes involved in human stress and toxicity plus 5 housekeeping genes (Table 1). PCR Array controls are included for PCR performance, genomic DNA contamination, and RNA quality verification.

Protein Analysis

We collected fasting participants' peripheral whole blood by venipuncture into citrate tubes on the first visit and spun it down at $2,000 \times g$ for 15 min at 4°C . We collected the plasma and followed the standard spike and recovery protocol to quantify IL-1 α protein using the Human IL-1 alpha/IL-1F1 Quantikine® ELISA Kit (R&D Systems, Inc., Minneapolis, MN).

Data and Statistical Analysis

We used SA Biosciences Web Analysis software (<http://www.sabiosciences.com>) to analyze the expression data; threshold cycle (Ct) cutoff was set at 35 cycles and boundary to 2-fold. Fold change ($2^{-\Delta\Delta\text{Ct}}$) was defined as the normalized gene expression ($2^{-\Delta\text{Ct}}$) in the test group divided by the normalized gene expression ($2^{-\Delta\text{Ct}}$) in the control group. Fold change values greater than 1 represent an upregulation, and the fold regulation is equal to the fold change. Fold-change values less than 1 represent a downregulation, and the fold regulation is the negative inverse of the fold change. Results are reported as fold regulation because it represents fold change values in a biologically meaningful way (Arikawa, Quellhorst, Han, Pan, & Yang, n.d.; McCarthy & Smyth, 2009). We considered dysregulation (up- or downregulation) greater than 2-fold to be significant for the purposes of this study. We analyzed clinical and demographic data with independent sample *t* tests using SPSS 15 (SPSS Inc., Chicago, IL) and set *p* values $< .05$ a priori as significant.

Results

Overall, the sample had PSS scores ranging from 0 to 25. Based on the aforementioned criteria, we defined high stress as a PSS score ≥ 19 and low stress as a PSS score ≤ 8 . We note participant demographics in Table 2. There were no significant differences in demographic characteristics between individuals in the high- and low-stress groups.

Gene Expression in Stress and CAP

Interleukin-1 alpha (*IL1A*) gene, which codes for the proinflammatory cytokine interleukin-1 alpha (IL-1 α), was upregulated by 2.58-fold (95% CI [0.88, 4.28]) in high-PSS females ($n = 6$) compared to low-PSS females ($n = 7$), upregulated by 1.35-fold (95% CI

[0.02, 2.68]) in high-PSS males ($n = 4$) compared to low-PSS males ($n = 9$), downregulated by -1.29 -fold (95% CI [0.18, 1.36]) in low-PSS females ($n = 7$) compared to low-PSS males ($n = 9$), upregulated by 1.48 -fold (95% CI [0.14, 2.82]) in high-PSS females ($n = 6$) compared to high-PSS males ($n = 4$) and downregulated by -1.19 -fold (95% CI [0.00001, 1.80]) in females with CAP ($n = 6$) compared to males with CAP ($n = 4$). *IL1A* was also upregulated by 1.91 -fold (95% CI [0.82, 3.00]) in high-PSS participants ($n = 10$) compared to low-PSS participants ($n = 16$), upregulated by 3.47 -fold (95% CI [1.14, 5.80]) in high-PSS participants with CAP ($n = 5$) compared to low-PSS participants with CAP ($n = 5$), and upregulated by 1.41 -fold (95% CI [0.33, 2.49]) in high PSS participants without CAP ($n = 5$) compared to low PSS participants without CAP ($n = 11$).

Protein Levels

There was a trend toward an increased IL-1 α level in high-PSS participants (5.00 ± 4.75 pg/ml) compared to low-PSS participants (4.38 ± 2.53 pg/ml, $p = .67$). There was a trend toward an increased IL-1 α level high-PSS participants with CAP (4.95 ± 4.92 pg/ml) compared to low-PSS participants with CAP (3.15 ± 2.39 pg/ml, $p = .48$). However, neither trend was statistically significant. There was no difference in IL-1 α levels between high-PSS participants without CAP and low-PSS participants without CAP. The coefficient of variation for the IL-1 α enzyme-linked immunosorbent assay (ELISA) was 3.34%.

Clinical Parameters

There was a significant difference ($p = .01$) in serum cortisol levels between high-PSS participants (8.56 ± 3.89 μ g/dl) and low-PSS participants (13.65 ± 5.00 μ g/dl). The ACTH and ACTH/cortisol ratios did not differ significantly between the groups.

Discussion

In this study, we examined the gene expression profiles of participants with either high or low levels of perceived stress and with or without the symptom of CAP. We defined dysregulation as a significant alteration in gene expression beyond 2-fold. Based on this definition, we found dysregulated *IL1A* expression in high-PSS females compared to low-PSS females but not in high-PSS males compared to low-PSS males. We also found dysregulated *IL1A* expression in high-PSS participants with CAP compared to low-PSS participants with CAP but not in high-PSS participants without CAP compared to low-PSS participants without CAP. *IL1A* codes for the protein IL-1 α , a member of the interleukin-1 cytokine family that is involved in various inflammatory and immune processes (Hauser et al., 1986). As part of the innate immune system, this cytokine can be produced by activated macrophages and can act as a chemotactic agent to attract neutrophils (Arend, 2002). Our findings of upregulation of *IL1A* in women with high PSS and in all participants with high PSS and CAP support previous data that found associations between circulating inflammatory cytokine levels and stress (Steptoe, Hamer, & Chida, 2007). Though IL-1 α protein levels were not significantly higher in participants with upregulated *IL1A* expression in the present study, there was a trend toward increased levels. Our finding of *IL1A* upregulation as an effect of CAP on stress provides evidence of an interaction between GI symptoms and stress. Therefore our finding of an upregulation of the gene coding the pro-inflammatory cytokine IL-1 α supports the hypothesis that the mechanism behind stress-related changes in GI symptoms is pro-inflammatory in nature.

Our finding of a significant difference in *IL1A* expression between females with high versus low perceived stress combined with the lack of a significant difference between males with high versus low stress offers a possible explanation of why females report symptoms associated with IBS twice as much as males (Drossman et al., 1993). Though this finding

may appear to contradict expectations raised by previous research showing that males have a greater autonomic response to acute stress than females (Prather et al., 2009), it is not surprising that our findings differ: in contrast to the previous study, we did not acutely induce stress nor did we stimulate cytokine production. Our findings in the present study, however, may be more generalizable to the natural phenomena in patients with GI symptoms. We also found that those with low stress had higher cortisol levels than those with high stress, another counterintuitive result. This finding may be related to a blunted stress response in those with CAP or to gender differences.

Limitations of this study include the use of the PSS because of the subjective nature of the tool and the absence of an absolute categorization of high and low stress. The use of peripheral blood as a means to assess the GI environment is another potential limitation. Use of intestinal biopsies for gene expression analysis might better focus analysis on localized interactions of stress and CAP. Another limitation of this study is the inclusion of participants of multiple races. In future studies, larger groups of participants of each race would enable comparisons between races. Finally, the genes we analyzed are a small subset of the human genome, and the results of this study could simply be one small piece of a larger genotypic and epigenetic scenario involving stress, gender, and GI symptom distress.

The results of this study contribute to the knowledge of the mechanism behind stress-related CAP symptoms and gender differences associated with functional GI disorders. Findings suggest a relationship between perceived stress and inflammatory biological alterations and provide further evidence supporting targeted management and treatment of stress in patients with GI symptoms. In particular, they suggest that implementation of biobehavioral interventions by nurses or health care practitioners for stress reduction in cases of functional GI disorders and CAP are warranted.

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Table 1

Human Stress and Toxicity PathwayFinder Gene Table

	1	2	3	4	5	6	7	8	9	10	11	12
A	ANXA5	ATM	BAX	BCL2L1	CASP1	CASP10	CASP8	CAT	CCL21	CCL3	CCL4	CCNC
B	CCND1	CCNG1	CDKN1A	CHEK2	CRYAB	CSF2	CXCL10	CYP1A1	CYP2E1	CYP7A1	DDB1	DDIT3
C	DNAJA1	DNAJB4	E2F1	EPHX2	ERCC1	ERCC3	ERCC3	FASLG	FMO1	FMO5	GADD45A	GDF15
D	GPX1	GSR	GSTM3	HMOX1	HSF1	HSPA1A	HSPA1L	HSPA2	HSPA4	HSPA5	HSPA6	HSPA8
E	HSPB1	HSP90AA2	HSP90AB1	HSPD1	HSPE1	HSPH1	IGFBP6	IL18	IL1A*	IL1B	IL6	LTA
F	MDM2	MIF	MT2A	NFKB1	NFKBIA	NOS2	PCNA	POR	PRDX1	PRDX2	PTGS1	RAD23A
G	RAD50	SERPINE1	SOD1	SOD2	TNF	TNFRSF1A	TNFSF10	TP53	UGT1A4	UNG	XRCC1	XRCC2
H	B2M	HPRT1	RPL13A	GAPDH	ACTB	HGDC	RTC	RTC	RTC	PPC	PPC	PPC

Note. Table shows all genes whose mRNA expression was analyzed for this study using RT² ProfilerTM PCR Array Human Stress and Toxicity (PAHS-003, SA Biosciences by Qiagen, Frederick, MD).

ANXA5 = Annexin A5; ATM = Ataxia telangiectasia mutated; BAX = BCL2-associated X protein; BCL2L1 = BCL2-like 1; CASP1 = Caspase 1; apoptosis-related cysteine peptidase (interleukin 1, beta, convertase); CASP10 = Caspase 10, apoptosis-related cysteine peptidase; CASP8 = Caspase 8, apoptosis-related cysteine peptidase; CAT = Catalase; CCL21 = Chemokine (C-C motif) ligand 21; CCL3 = Chemokine (C-C motif) ligand 3; CCL4 = Chemokine (C-C motif) ligand 4; CCNC = Cyclin D1; CCNG1 = Cyclin G1; CDKN1A = Cyclin-dependent kinase inhibitor 1A (p21, Cip1); CHEK2 = CHK2 checkpoint homolog (S. pombe); CRYAB = Crystallin, alpha B; CSF2 = Colony stimulating factor 2 (granulocyte-macrophage); CXCL10 = Chemokine (C-X-C motif) ligand 10; CYP1A1 = Cytochrome P450, family 1, subfamily A, polypeptide 1; CYP2E1 = Cytochrome P450, family 2, subfamily E, polypeptide 1; CYP7A1 = Cytochrome P450, family 7, subfamily A, polypeptide 1; DDB1 = Damage-specific DNA binding protein 1, 127 kDa; DDIT3 = DNA-damage-inducible-transcript 3; DNAJA1 = DnaJ (Hsp40) homolog, subfamily A, member 1; DNAJB4 = DnaJ (Hsp40) homolog, subfamily B, member 4; E2F1 = E2F transcription factor 1; EGR1 = Early growth response 1; EPHX2 = Epoxide hydrolase 2, cytoplasmic; ERCC1 = Excision repair cross-complementing rodent repair deficiency, complementation group 1 (includes overlapping antisense sequence); ERCC3 = Excision repair cross-complementing rodent repair deficiency, complementation group 3 (xeroderma pigmentosum group B complementing); FASLG = Fas ligand (TNF superfamily, member 6); FMO1 = Flavin containing monooxygenase 1; FMO5 = Flavin containing monooxygenase 5; GADD45A = Growth arrest and DNA-damage-inducible, alpha; GDF15 = Growth differentiation factor 15; GPX1 = Glutathione peroxidase 1; GSR = Glutathione S-transferase mu 3 (brain); HMOX1 = Heme oxygenase (decycling) 1; HSF1 = Heat shock transcription factor 1; HSPA1A = Heat shock 70 kDa protein 1A; HSPA1L = Heat shock 70 kDa protein 1-like; HSPA2 = Heat shock 70 kDa protein 2; HSPA4 = Heat shock 70 kDa protein 4; HSPA5 = Heat shock 70 kDa protein 5 (glucose-regulated protein, 78 kDa); HSPA6 = Heat shock 70 kDa protein 6 (HSP70B^{*}); HSPA8 = Heat shock 70 kDa protein 8; HSPB1 = Heat shock 27 kDa protein 1; HSP90AA2 = Heat shock 90 kDa alpha (cytosolic), class A member 2; HSP90AB1 = Heat shock 90 kDa alpha (cytosolic), class B member 1; HSPD1 = Heat shock 60 kDa protein 1 (chaperonin); HSPE1 = Heat shock 10 kDa protein 1 (chaperonin 10); HSPH1 = Heat shock 105kDa/110 kDa protein 1; IGFBP6 = Insulin-like growth factor binding protein 6; IL18 = Interleukin 18 (interferon-gamma-inducing factor); IL1A = Interleukin 1, alpha; IL1B = Interleukin 1, beta; IL6 = Interleukin 6 (interferon, beta 2); LTA = Lymphotoxin alpha (TNF superfamily, member 1); MDM2 = Mdm2 p53 binding protein homolog (mouse); MIF = Macrophage migration inhibitory factor (glycosylation-inhibiting factor); MT2A = Metallothionein 2A; NFKB1 = Nuclear factor of kappa light polypeptide gene enhancer in B-cells 1; NFKBIA = Nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, alpha; NOS2 = Nitric oxide synthase 2, inducible; PCNA = Proliferating cell nuclear antigen; POR = P450 (cytochrome) oxidoreductase; PRDX1 = Peroxiredoxin 1; PRDX2 = Peroxiredoxin 2; PTGS1 = Prostaglandin-endoperoxide synthase 1 (prostaglandin G/H synthase and cyclooxygenase); RAD23A = RAD23 homolog A (*S. cerevisiae*); RAD50 = RAD50 homolog (*S. cerevisiae*); SERPINE1 = Serpin peptidase inhibitor, clade E (nexin), plasminogen activator inhibitor type 1, member 1; SOD1 = Superoxide dismutase 1, soluble; SOD2 = Superoxide dismutase 2, mitochondrial; TNF = Tumor necrosis factor (TNF superfamily, member 2); TNFRSF1A = Tumor necrosis factor receptor superfamily, member 1A; TNFSF10 = Tumor necrosis factor (ligand) superfamily, member 10; TP53 = Tumor protein p53; UGT1A4 = UDP glucuronosyltransferase 1 family, polypeptide A4; UNG = Uracil-DNA glycosylase; XRCC1 = X-ray repair complementing defective repair in Chinese hamster cells 1; XRCC2 = X-ray repair complementing defective repair in Chinese hamster cells 2; B2M = Beta-2-microglobulin; HPRT1 = Hypoxanthine phosphoribosyltransferase 1; RPL13A = Ribosomal protein L13a; GAPDH = Glyceraldehyde-3-phosphate dehydrogenase; ACTB = Actin, beta; HGDC = Human genomic DNA contamination; RTC = Reverse transcription control; PPC = Positive PCR control.

* IL1A expression differed significantly between the groups ($p < .05$).

Table 2

Sample Characteristics

Variable	Overall (N = 26)	High PSS (n = 10)	Low PSS (n = 16)	p Value
Gender, male (n)	13	4	9	—
Age (years)	26.50 ± 7.02	26.30 ± 6.62	26.62 ± 7.47	0.91
PSS score	10.73 ± 8.72	21.20 ± 2.04	4.19 ± 2.32	< 0.001
CAP positive (n)	10	5	5	0.34
ACTH (pg/mL)	16.22 ± 5.29	15.45 ± 3.76	16.70 ± 6.13	0.57
Cortisol (µg/dL)	11.69 ± 5.18	8.56 ± 3.89	13.65 ± 5.00	0.01 *
ACTH/cortisol ratio	1.79 ± 1.22	2.35 ± 1.59	1.44 ± 0.79	0.06

Note. Values reported as mean ± *SD*, unless otherwise noted. The high-PSS group had PSS scores 19; the low-PSS group had PSS scores 8. PSS = perceived stress scale; CAP = chronic abdominal pain; ACTH = adrenocorticotrophic hormone.

* *p* value < .05 considered significant.