

# Randomized, Double-Blind Placebo-Controlled Trial to Assess the Effect of Probiotics on Irritable Bowel Syndrome in Veterans With Gulf War Illness

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**Background:** Many veterans who served in Operation Desert Storm (August 1990 to March 1991) experienced a complex of symptoms of unknown etiology called Gulf War illness (GWI), which significantly impacts the health and quality of life (QOL) and may have contributed to irritable bowel syndrome (IBS).

**Methods:** We performed a prospective, double-blind placebo-controlled study to determine the efficacy of the multistrain De Simone Formulation probiotic containing 8 strains of bacteria on symptoms of IBS and GWI. Veterans of Operation Desert Storm who had IBS and  $\geq 2$  nonintestinal symptoms of GWI were included. The primary study endpoint was change in bowel symptom score. The secondary endpoints were

mean change in symptoms, QOL, and extra-intestinal and posttraumatic stress disorder (PTSD) symptoms.

**Results:** A total of 101 Gulf War veterans with IBS and GWI were screened at the Veteran Affairs Medical Center in Salt Lake City, Utah. The study was completed by 53 veterans; 47 (89%) were male with a mean (SD) age of 55 (8) years. The probiotic did not improve IBS symptoms or other extra-intestinal symptoms common to IBS and GWI.

**Conclusions:** Our study did not demonstrate statistically significant improvement in IBS symptoms or QOL after treatment with the probiotic. We also did not find any improvement in symptoms of GWI or PTSD.

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About 700,000 US military personnel were deployed in Operation Desert Storm (August 1990 to March 1991).<sup>1</sup> Almost 30 years since the war, a large number of these veterans continue to experience a complex of symptoms of unknown etiology called Gulf War illness (GWI), which significantly affects health and quality of life (QOL). The lack of clear etiology of the illness has impaired research to find specific treatments and has further exacerbated the stress among veterans. GWI typically includes a mixture of chronic headache, cognitive difficulties, widespread pain, unexplained fatigue, memory and concentration problems, as well as chronic respiratory and gastrointestinal (GI) symptoms.<sup>2</sup> Abdominal pain and alteration of bowel habits are also symptoms typical of irritable bowel syndrome (IBS). It has been estimated that IBS occurs in up to 30% of Gulf War veterans.<sup>3</sup>

The etiology of IBS is unknown. Possible mechanisms include visceral hypersensitivity, altered gut motor function, aberrant brain-gut interaction, and psychological factors, perhaps with a genetic predisposition.<sup>4</sup> Gastroenteritis has been reported as a triggering mechanism in up to one-

third of patients with IBS.<sup>5</sup> Gastroenteritis can alter the gut microbiota and has been reported to be a significant risk factor for the development of IBS.<sup>6</sup> In one study of Operation Desert Shield soldiers, > 50% of military personnel developed acute gastroenteritis while on duty.<sup>7</sup> A high prevalence of extra-intestinal symptoms also has been reported, including fatigue, headache, joint pains, and anxiety, in Gulf War veterans with IBS. These extra-intestinal symptoms of IBS are consistent with the reported GWI symptoms. Change in gut microbiota also has been associated with many of the extra-intestinal symptoms of IBS, especially fatigue.<sup>8,9</sup> Gut microbiota are known to change with travel, stress, and a change in diet, all potential factors that are relevant to Gulf War veterans. This would suggest that an imbalance in the gut microbiota, ie, dysbiosis, may play a role in the pathogenesis of both IBS and GWI. Dysbiosis could be a risk factor for or alternatively a consequence of GWI.

A systematic review highlighted the heterogeneity of the gut microbiota in patients with IBS.<sup>10</sup> Overall, *Enterobacteriaceae*, *Lactobacillaceae*, and *Bacteroides* were increased, whereas *Clostridiales*, *Faecalibacterium*, and

*Bifidobacterium* were decreased in patients with IBS compared with controls. Gut microbiota also has been associated with cognitive changes, anxiety, and depression—symptoms associated with IBS and are part of the GWI.

If altered gut microbiota contributes to the etiopathogenesis of IBS, its restoration of with probiotics should help. Probiotics are live organisms that when ingested may improve health by promoting the growth of naturally occurring flora and establishing a healthy gut flora. Probiotics have several mechanisms of actions. Probiotics work in the lumen of the gut by producing antibacterial molecules and enhancing the mucosal barrier.<sup>11</sup> Probiotics also may produce metabolic compounds that alter the intestinal microbiota and improve intestinal barrier function.<sup>12</sup> Probiotics also have been shown to activate receptors in the enteric nervous system with the potential to promote pain relief in the setting of visceral hyperalgesia.<sup>13,14</sup> The anti-inflammatory properties of probiotics potentially could modulate the basic pathophysiology of IBS and improve motility, visceral hypersensitivity, and brain-gut interaction.<sup>15</sup> Furthermore, significant gut dysbiosis has been shown with GWI; suggesting that probiotics may have a role in its management.<sup>16,17</sup>

Probiotics have not been studied in Gulf War veterans with IBS. We performed a prospective, double-blind placebo-controlled study to determine the efficacy of a commercially available probiotic containing 8 strains of bacteria (De Simone Formulation; formally known as VSL#3 and Visbiome) on symptoms of IBS and GWI. This probiotic was selected as the overall literature suggested benefit of combination probiotics in IBS, and VSL#3 has been shown to be efficacious in ulcerative colitis and microscopic colitis.<sup>18-20</sup>

## METHODS

Veterans who served in Operation Desert Storm (August 1990 to March 1991) and enrolled at the George E. Wahlen Veterans Affairs (VA) Medical Center (GEWVAMC), Salt Lake City, Utah, were eligible for the study. The inclusion criteria were: veterans aged  $\geq 35$  years;  $\geq 2$  nonintestinal GWI symptoms (eg, fatigue, joint pains, insomnia, general stiffness, and headache);

**TABLE 1** Demographics and Baseline Symptoms

Characteristics	Probiotics (n = 24)	Placebo (n = 29)	P value
Male sex No. (%)	22 (92)	25 (86)	.68
Age, mean (SD), y	54 (7)	56 (9)	.38
Brief Symptom Inventory 18, No. (%)	23 (17)	30 (18)	.20
Somatization subscale	8 (6)	9 (6)	.48
Depression subscale	7 (6)	10 (8)	.09
Anxiety subscale	9 (8)	11 (7)	.33

IBS diagnosis based on the Rome III criteria; IBS symptoms  $> 6$  months; normal gross appearance of the colonic mucosa; negative markers for celiac disease and inflammatory bowel disease (IBD); normal thyroid function; and serum calcium levels.<sup>21</sup> Those who had a clinically significant cardiac, pulmonary, hepatic or renal dysfunction; history of/or presence of systemic malignancy; current evidence of celiac disease or IBD; unstable/significant psychiatric disease; recent change in GI medications; current pregnancy; or use of antibiotics or probiotics within the past 1 month were excluded. Subjects were enrolled from a list of veterans with GWI from the GEWVAMC Gulf War registry; referrals to gastroenterology clinics for IBS from internal medicine clinics; and posted advertisements.

## Protocol

After written informed consent was obtained, each veteran was verified to have IBS and  $\geq 2$  GWI symptoms. All veterans had the following tests and panels: complete blood count, erythrocyte sedimentation rate, serum comprehensive metabolic panel, thyroid-stimulating hormone, tissue transglutaminase, stool test for ova and parasite, giardia antigen, and clostridia toxins to exclude organic cause of GI symptoms. Colonoscopy was performed in all veterans to exclude IBD, and to rule out microscopic or lymphocytic colitis.

Randomization was computer generated and maintained by the study pharmacist so that study personnel and patients were blinded to the trial groups. All investigators were blinded and allocation was concealed. The medication was supplied in a numbered container by the pharmacist after patient enrollment. After a 2-week run-in period, veterans were randomized (1:1) to receive either

**TABLE 2** IBS Symptoms and Change With Treatment

Symptom scales	Probiotic, mean (SEM) (n = 24)			Placebo, mean (SEM) (n = 29)			P value
	Baseline	End	Change	Baseline	End	Change	
Abdominal pain <sup>a</sup>	35 (6)	37 (6)	2 (5)	39 (5)	38 (5)	0 (6) <sup>b</sup>	.75
Bloating <sup>a</sup>	39 (7)	37 (6)	-2 (6)	42 (6)	36 (5)	-6 (7)	.74
Stool frequency <sup>a</sup>	3.5 (0.4)	3.1 (0.4)	-0.4 (0.3)	3.5 (0.3)	3.2 (0.4)	-0.3 (0.2)	.76
Stool consistency <sup>a</sup>	4.9 (0.2)	4.6 (0.3)	-0.3 (0.4)	5.1 (0.3)	4.7 (0.3)	-0.4 (0.4)	.78
Diarrhea severity <sup>a</sup>	58 (6)	41 (6)	-18 (6) <sup>b</sup>	47 (6)	41 (5)	-6 (5)	.13
Constipation <sup>a</sup>	27 (6)	21 (5)	-5 (5) <sup>b</sup>	24 (5)	27 (5)	-2 (5) <sup>b</sup>	.29
Bowel habit satisfaction <sup>a</sup>	46 (7)	30 (6)	-16 (7)	37 (6)	41 (6)	4 (9)	.09
IBS interfering with life <sup>a</sup>	64 (5)	60 (6)	-4 (7)	65 (4)	60 (5)	-5 (4)	.84
Bowel symptom total	41 (4)	33 (3)	-8 (3)	38 (3)	36 (3)	-1 (3) <sup>b</sup>	.13
PTSD scale	43.7 (4.5)	44.4 (4.8)	0.7 (1.5)	50.8 (3.5)	52.0 (3.3)	1.3 (1.3) <sup>b</sup>	.77

Abbreviation: IBS, irritable bowel syndrome; PTSD, posttraumatic stress disorder.

<sup>a</sup>100-point scale.

<sup>b</sup>Discrepancies are due to rounding errors.

1 sachet of probiotic (De Simone Formulation; formally known as VSL#3 and Visbiome) or placebo once daily for 8 weeks.

Each probiotic packet contains 900 billion probiotic bacteria per sachet.<sup>11</sup> This formulation contained 8 viable strains of bacteria: 4 strains of *Lactobacillus* (*L. acidophilus*, *L. plantarum*, *L. paracasei*, *L. delbrueckii subsp. bulgaricus*); 3 strains of Bifidobacteria (*Bifidobacterium breve*, *B. lactis*, *B. infantis*); and 1 strain of *Streptococcus thermophilus*. This formulation had been commercialized and studied as VSL#3 and is currently available in the United States under the Visbiome trade name. While branding changed during the study, the formulation did not. The investigational medicine (VSL#3, Visbiome, and placebo) were shipped from the manufacturer Dupont/Danisco in Madison, Wisconsin. The subjects received placebo or probiotic (VSL#3/Visbiome) and both were identical in appearance. The medication was supplied in a numbered container by the pharmacist after patient enrollment.

### Measures

Veterans completed the bowel disease questionnaire to record baseline bowel habits.<sup>22</sup> All veterans recorded daily bowel symptoms to confirm the presence of IBS during the 2-week pretreatment period, at baseline,

and at the end of the 8-week treatment. The symptoms assessed included severity of abdominal pain (0, none to 100, severe); severity of bloating (0, none to 100, severe); stool frequency; Bristol stool scale (1, very hard to 7, watery); severity of diarrhea (0, none to 100, severe); severity of constipation (0, none to 100, severe); satisfaction with bowel habits (0, none to 100, severe); and IBS affecting or interfering with life (0, none to 100, severe). The bowel symptom score is the sum of the 5 symptom scores.<sup>23,24</sup>

IBS-specific QOL (IBS-QOL) was recorded at baseline and at the end of treatment.<sup>25</sup> The IBS-QOL consists of a 34-item validated disease-specific questionnaire that measures 8 domains relevant to subjects with IBS: dysphoria, interference with activity, body image, health worry, food avoidance, social reaction, sexual life, and relationships. We used the Somatic Symptom Checklist to detect the following extra-intestinal symptoms that are common among veterans with GWI: headache, backache, wheeziness, insomnia, bad breath, fatigue, general stiffness, dizziness, weakness, sensitivity to hot and cold, palpitation, and tightness in chest. Subjects rated symptoms on a scale of 1 to 5: how often (1, none; 2, monthly; 3, once weekly; 4, several times weekly; 5, daily), and how bothersome (1, not at all to 5, extremely).<sup>26</sup>

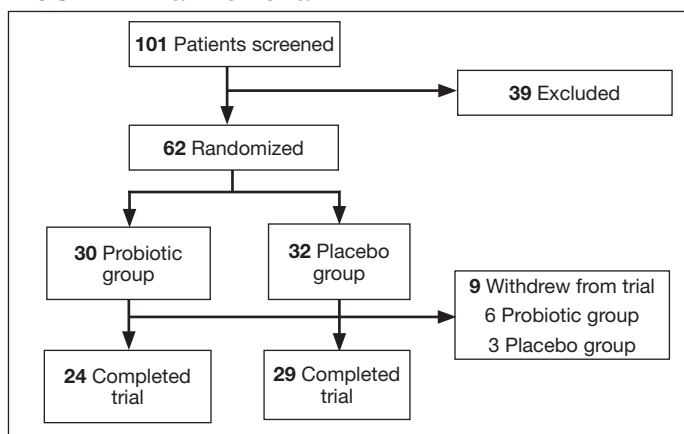
Subjects completed the Posttraumatic Stress Disorder (PTSD) Checklist–Military, which is specific to military experience with 17 items on a 1 to 5 scale (1, not at all to 5, extremely). Scores were summed to produce a total symptom severity score (range, 17-85).<sup>27</sup> Subjects also completed the Brief Symptom Inventory 18 (BSI-18) during the baseline evaluation.<sup>28</sup> BSI-18 measures subjects' reported overall psychological distress. It assesses 3 symptoms dimensions (somatization, depression, and anxiety) and a global severity index. The raw scores were transferred to normative T scores based on samples of nonpatient normal men and women.

Symptom data were compared after 8 weeks of treatment. The primary study endpoint was change in bowel symptom score. The secondary endpoints were mean change in symptoms, QOL, extra-intestinal symptoms, and PTSD score. The study was approved by the Salt Lake City Veterans Affairs Medical Center and the University of Utah Institutional Review Board and registered in ClinicalTrials.gov (NCT03078530).

### Statistical Methods

Comparisons of the probiotic vs placebo groups for demographic variable were analyzed using a 2-sample *t* test for continuous variables, and with a  $\chi^2$  test or Fisher exact test for categorical variables. The primary and secondary outcome variables were recorded daily for 2 weeks as pretreatment baseline and for 2 weeks at the end of treatment. These symptoms were recorded as ordered categorical variables, which were then averaged across the week to produce a continuous measurement for statistical analysis. For the primary outcome of GI symptoms, posttreatment comparisons were made between the study groups using a 2-sample *t* test of the baseline vs posttreatment values. All *P* values were calculated for 2-sided comparisons. The planned sample size in our study protocol was to recruit 40 individuals per group in order to achieve 80% power to detect a 30% improvement between baseline and end of treatment in the primary bowel symptom score. This study recruited 53 subjects. With this sample size, the study had 80% power to detect a 0.8 SD in any of the outcomes.

**FIGURE 1** Trial Flowchart

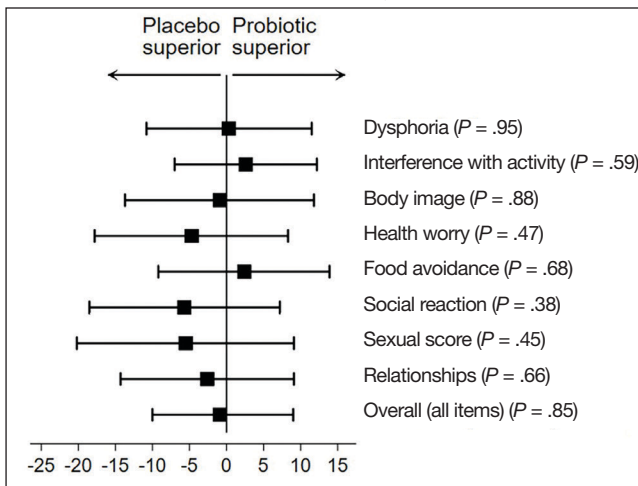


### RESULTS

We screened 101 veterans with IBS and GWI; 39 veterans did not fulfill the inclusion/exclusion criteria, 22 declined to participate or did not complete the screening questionnaires and tests, and 9 were lost to follow-up. Sixty-two participants were randomized in a double-blind placebo-controlled study design; 9 dropped out before the end of the study. Data were analyzed from 53 veterans who completed the study, 29 in the placebo group and 24 in the probiotic group (Figure 1). The cohort was primarily male with a mean (SD) age of 55 (8) years (range, 42-73) (Table 1).

Overall, the treatment was well tolerated. All subjects were contacted every 2 weeks during the study to check for adverse effects, but no serious events were reported. There were no differences at baseline in any of the BSI-18 subscale scores in veterans between the groups. There was a greater mean (SEM) improvement of diarrhea severity in the probiotic group compared with the placebo group: 18 (6), a 31% improvement, vs 6 (5), a 13% improvement, respectively; however, the difference was not statistically significant ( $P = .13$ ) (Table 2). There also was a greater mean (SEM) improvement in satisfaction of bowel habits in the probiotic group compared with the placebo group: 16 (7), a 35% improvement vs 4 (9), an 8% worsening; this also was not statistically significant ( $P = .09$ ). There was no difference in the change of IBS-QOL before and after treatment in either group (Figure 2). There was no improvement in any of the symptoms of GWI (all  $P \geq .06$ ) (Appendix).

**FIGURE 2** IBS-Specific Quality-of-Life Measure<sup>a</sup>



Abbreviation: IBS, irritable bowel syndrome.  
<sup>a</sup>Univariable mean differences with 2-sided 95% CI.

**DISCUSSION**

GWI is a complex multisystem illness of unknown etiology. There was high prevalence of diarrhea during deployment, and veterans were exposed to several physical, environmental, and mental stresses of the war.<sup>3</sup> A change in gut microbiota can occur during deployment due to diet changes, environmental and physical stress, and GI infections.<sup>29</sup> These changes would suggest that manipulation of gut microbiota might offer a new modality of treatment of IBS and GWI. We evaluated the effect of a high-potency multistrain probiotic in veterans with IBS and GWI. We did not detect any statistically significant differences between the probiotic and placebo groups on bowel symptom score and individual symptoms of IBS and on QOL. Also, there was no improvement for the other symptoms of GWI. To our knowledge, this is the first study evaluating the effect of probiotics in veterans with IBS and GWI. Our results are consistent with the literature on probiotics and IBS.

The probiotic formulation used in our study has been evaluated in patients with IBS previously. Kim and colleagues found that after 8 weeks of treatment of patients with diarrhea-predominant IBS with VSL#3, there was improvement in bloating, but no effect was found on abdominal pain, gas, or urgency.<sup>30</sup> A subsequent study by the same investigators on patients with all types

of IBS found that VSL#3 showed no effect on abdominal pain, stool frequency and consistency, or on bloating, but there was improvement in flatulence.<sup>31</sup> Another study that evaluated the effect of VSL#3 on symptoms of diarrhea-predominant IBS and QOL found improvement in IBS symptoms from baseline in both the probiotic and the placebo groups, but the difference between the 2 groups was not statistically significant.<sup>32</sup> Similarly, Wong and colleagues performed a double-blind, placebo-controlled mechanistic study to evaluate the effect of VSL#3. They found improvement in bowel symptom score, abdominal pain intensity, and satisfaction with bowel habits with both the VSL#3 and placebo group but similar to our study, the differences were not statistically significant.

Several reviews have evaluated the efficacy of probiotics for IBS. A 2010 review found evidence that probiotics trended toward improved IBS symptoms compared with placebo.<sup>33</sup> The 2014 follow-up by the same authors demonstrated that overall, probiotics improved global symptoms of IBS and multistrain probiotics were more effective.<sup>20</sup> A third meta-analysis from the same group found evidence that multistrain probiotics seemed to have a beneficial effect but could not definitively conclude that probiotics are efficacious in improving IBS symptoms.<sup>34</sup> Other authors also have seen inconsistent effects of probiotics compared with placebo on global symptoms, abdominal pain, and bloating after performing systematic reviews of the literature.<sup>35-38</sup> Although several reviews support that multistrain probiotics are more effective, they fail to conclude which combinations are more efficacious.

The effect of probiotics on QOL has not been investigated by many studies.<sup>37</sup> In our study, we did not find significant improvement in QOL in the probiotic group, which is in line with 2 previous studies that showed no effect on IBS QOL of VSL#3 vs placebo.<sup>32,39</sup> Most of the research reports that multistrain probiotics are more effective than using a single strain.<sup>34,35,40</sup> *Bifidobacterium* and *Lactobacillus* are the most commonly used bacteria in the multistrain probiotics that have shown their positive effect on IBS.<sup>35,41</sup> The probiotic used in our study contained other species along with these 2 microorganisms.

The dose and duration of treatment of



probiotics also has been debated. In one meta-analysis, the investigators found that studies of  $\geq 8$  weeks were more likely to show a positive effect; 4 of the 7 studies with statistically significant improvement in IBS symptoms were longer than 8 weeks.<sup>35</sup> However, another meta-analysis based on 35 randomized controlled trials found that there was not a statistically significant difference between groups treated for  $> 4$  weeks vs  $< 4$  weeks.<sup>42</sup> In addition, another meta-analysis of VSL#3 on IBS in children and adults also found no difference in results based on the duration of treatment of probiotics.<sup>43</sup> Similar to our study, 3 other studies of VSL#3 treated patients for 8 weeks and found no statistically significant effect.<sup>30-32</sup> In the past, VSL#3 has been used at dosages of 450 or 900 billion bacteria per day.

An individual's response to probiotics may depend on the subtype of IBS. However, most of the studies, like ours, included groups of all subtypes. It may be that probiotics are more effective in patients with moderate-to-severe symptoms. Most of our patients had milder symptoms, and we cannot discount how subjects with more severe disease may have responded to the drug. Interestingly, one study demonstrated that *Lactobacillus* was more effective in patients with moderately severe abdominal pain compared with mild symptoms.<sup>44</sup>

In our study, the probiotic did not improve PTSD symptoms or other extra-intestinal symptoms common in IBS and GWI. Similar to our study, Wong and colleagues did not find significant improvement of psychological and sleep scores after treatment with VSL#3.<sup>6</sup> Similarly, there is evidence that alteration in gut microbiota is associated with health and diseases, but what specific alterations occur and whether they can be improved with probiotics remains unknown.<sup>45</sup>

### Limitations

The inconsistent response to probiotics in various studies may be due to IBS heterogeneity. Furthermore, there are demographic differences between Gulf War veterans and patients enrolled in other studies: Gulf War veterans are predominantly male, many were deployed abroad and had a history of gastroenteritis during deployment, and were exposed to stressful situations.<sup>46</sup> These

factors may be involved in triggering or maintaining IBS in Gulf War veterans. A further limitation of our randomized trial is the relatively small sample size.

### CONCLUSIONS

This study did not demonstrate statistically significant improvement in symptoms of IBS or improvement in QOL after treatment with a multistrain probiotic. We also did not find any improvement in symptoms of GWI or PTSD. There was no difference in psychological scores between the placebo and treatment groups, and it is unlikely that psychological factors confounded the response to treatment in this study.

The effectiveness of a probiotic may depend on the baseline gut microbiome of the individual and depend on the strain, amount, and frequency of bacteria used. A lack of response of the probiotics does not exclude gut viruses and fungi having a role in exacerbating GWI symptoms. It is also possible that the bacteria present or the dose of the probiotic used was not sufficient to improve symptoms. So far, the definitive benefit of probiotics has been demonstrated for only a few preparations, and none are approved by the US Food and Drug Administration for any disease. More research is needed to determine whether probiotics have any role in the treatment of IBS and GWI.

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### Disclaimer

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### Ethics and consent

The study was approved by the Salt Lake City Veterans Affairs Medical Center and the University of Utah Institutional Review Board. The study was registered in ClinicalTrials.gov (NCT03078530).

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## APPENDIX Mean Change for Posttraumatic Stress Disorder and Extra-Intestinal Symptoms Checklist<sup>a</sup>

Symptoms	Mean change (SEM)		
	Probiotic (n = 24)	Placebo (n = 29)	P value
Headache			
Frequency	-0.2 (0.2)	-0.4 (0.2)	.48
Level	0.1 (0.2)	0.2 (0.2)	.79
Backache			
Frequency	0.6 (0.3)	0 (0.1)	.03
Level	0.4 (0.2)	0 (0.2)	.17
Asthma			
Frequency	0.0 (0.1)	0.2 (0.1)	.44
Level	0.1 (0.1)	-0.1 (0.2)	.45
Trouble breathing			
Frequency	-0.1 (0.3)	-0.1 (0.2)	.98
Level	-0.1 (0.2)	-0.3 (0.2)	.35
Insomnia			
Frequency	0.0 (0.2)	0.2 (0.2)	.68
Level	0.0 (0.3)	-0.1 (0.2)	.72
Fatigue			
Frequency	0.0 (0.1)	0.2 (0.2)	.41
Level	-0.3 (0.1)	-0.1 (0.2)	.48
Stiffness			
Frequency	0.1 (0.2)	0.0 (0.3)	.81
Level	0.3 (0.2)	-0.2 (0.2)	.13
Heart palpitation			
Frequency	0.3 (0.2)	0.1 (0.2)	.40
Level	0.1 (0.2)	0.0 (0.3)	.80
Joint pain			
Frequency	0.3 (0.2)	-0.3 (0.2)	.06
Level	0.2 (0.3)	-0.2 (0.2)	.17
Eye pain			
Frequency	0.1 (0.2)	0.0 (0.2)	.70
Level	-0.1 (0.2)	0.0 (0.2)	.77
Dizziness			
Frequency	0.0 (0.2)	0.0 (0.2)	.90
Level	0.0 (0.2)	0.2 (0.2)	.49
Weakness			
Frequency	0.2 (0.3)	0.4 (0.2)	.52
Level	0.2 (0.3)	0.0 (0.2)	.51
Nervousness/shaking			
Frequency	-0.2 (0.2)	0.2 (0.2)	.17
Level	-0.4 (0.2)	0.1 (0.2)	.17
Hot/cold spell			
Frequency	-0.1 (0.2)	-0.2 (0.2)	.93
Level	-0.2 (0.3)	0.0 (0.3)	.71
High blood pressure			
Frequency	-0.1 (0.2)	-0.1 (0.2)	.96
Level	0.0 (0.2)	0.0 (0.3)	.76
Anxious/fearful/afraid			
Frequency	-0.1 (0.2)	0.3 (0.2)	.15
Level	-0.1 (0.2)	0.0 (0.2)	.87

<sup>a</sup>Not all study participants answered every questions.