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Sick building syndrome (SBS) and exposure to water-damaged buildings: Time series study, clinical trial and mechanisms

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Abstract

Occupants of water-damaged buildings (WDBs) with evidence of microbial amplification often describe a syndrome involving multiple organ systems, commonly referred to as "sick building syndrome" (SBS), following chronic exposure to the indoor air. Studies have demonstrated that the indoor air of WDBs often contains a complex mixture of fungi, mycotoxins, bacteria, endotoxins, antigens, lipopolysaccharides, and biologically produced volatile compounds. A case-series study with medical assessments at five time points was conducted to characterize the syndrome after a double-blinded, placebo-controlled clinical trial conducted among a group of study participants investigated the efficacy of cholestyramine (CSM) therapy. The general hypothesis of the time series study was that chronic exposure to the indoor air of WDBs is associated with SBS. Consecutive clinical patients were screened for diagnosis of SBS using criteria of exposure potential, symptoms involving at least five organ systems, and the absence of confounding factors. Twenty-eight cases signed voluntary consent forms for participation in the time-series study and provided samples of microbial contaminants from water-damaged areas in the buildings they occupied. Twenty-six participants with a group-mean duration of illness of 11 months completed examinations at all five study time points. Thirteen of those participants also agreed to complete a double-blinded, placebo-controlled clinical trial. Data from Time Point 1 indicated a group-mean of 23 out of 37 symptoms evaluated; and visual contrast sensitivity (VCS), an indicator of neurological function, was abnormally low in all participants. Measurements of matrix metalloproteinase 9 (MMP9), leptin, alpha melanocyte stimulating hormone (MSH), vascular endothelial growth factor (VEGF), immunoglobulin E (IgE), and pulmonary function were abnormal in 22, 13, 25, 14, 1, and 7 participants, respectively. Following 2 weeks of CSM therapy to enhance toxin elimination rates, measurements at Time Point 2 indicated group-means of 4 symptoms with 65% improvement in VCS at mid-spatial frequency—both statistically significant improvements relative to Time Point 1. Moderate improvements were seen in MMP9, leptin, and VEGF serum levels. The improvements in health status were maintained at Time Point 3 following a 2-week period during which CSM therapy was suspended and the participants avoid re-exposure to the WDBs. Participants reoccupied the respective WDBs for 3 days without CSM therapy, and all participants reported relapse at Time Point 4. The group-mean number of symptoms increased from 4 at Time Point 2 to 15 and VCS at mid-spatial frequency declined by 42%, both statistically significant differences relative to Time Point 2. Statistically significant differences in the group-mean levels of MMP9 and leptin relative to Time Point 2 were also observed. CSM therapy was reinstated for 2 weeks prior to assessments at Time Point 5. Measurements at Time Point 5 indicated group-means of 3 symptoms and a 69% increase in VCS, both results statistically different from those at Time Points 1 and 4. Optically corrected Snellen Distance Equivalent visual acuity scores did not vary significantly over the course of the study. Group-mean levels of MMP9 and leptin showed statistically significant improvement at Time Point 5 relative to Time Points 1 and 4, and the proportion of participants with abnormal VEGF levels was significantly lower at Time Point 5 than at Time Point 1. The number of participants at Time Point 5 with abnormal levels of MMP9, leptin, VEGF, and pulmonary function were 10, 10, 9, and 7, respectively. The level of IgE was not re-measured because of the low incidence of abnormality at Time Point 1, and MSH was not re-measured because previously published data indicated a long time course for MSH improvement. The results from the time series study supported the general study hypothesis that exposure to the indoor air of WDBs is associated with SBS. High levels of MMP9 indicated that exposure to the complex mixture of substances in the indoor air of the WDBs triggered a pro-inflammatory cytokine response. A model describing modes of

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action along a pathway leading to biotoxin-associated illness is presented to organize current knowledge into testable hypotheses. The model links an inflammatory response with tissue hypoxia, as indicated by abnormal levels of VEGF, and disruption of the proopiomelanocortin pathway in the hypothalamus, as evidenced by abnormalities in leptin and MSH levels. Results from the clinical trial on CSM efficacy indicated highly significant improvement in group-mean number of symptoms and VCS scores relative to baseline in the 7 participants randomly assigned to receive 2 weeks of CSM therapy, but no improvement in the 6 participants assigned placebo therapy during that time interval. However, those 6 participants also showed a highly significant improvement in group-mean number of symptoms and VCS scores relative to baseline following a subsequent 2-week period of CSM therapy. Because the only known benefit of CSM therapy is to enhance the elimination rates of substances that accumulate in bile by preventing re-absorption during enterohepatic re-circulation, results from the clinical trial also supported the general study hypothesis that SBS is associated with exposure to WDBs because the only relevant function of CSM is to bind and remove toxigenic compounds. Only research that focuses on the signs, symptoms, and biochemical markers of patients with persistent illness following acute and/or chronic exposure to WDBs can further the development of the model describing modes of action in the biotoxin-associated pathway and guide the development of innovative and efficacious therapeutic interventions.

Keywords: Sick building syndrome; Water-damaged buildings; Visual contrast sensitivity; Cholestyramine; Mold; Fungi; Mycotoxins

1. Introduction

The hypothesis that chronic exposure to the indoor environments of water-damaged buildings (WBD) causes a multi-system illness, often referred to as "sick building syndrome" (SBS), remains controversial [2–4,17,24,27]. Reviews by the authors and the California Research Board of the scientific literature on studies investigating the association between WBD exposure and SBS identified many studies that supported the hypothesis [95,110]. The WDB environment supported microbial amplification in the indoor environment, and indoor air contained a complex mixture of substances, including toxigenic fungi, bacteria, mycotoxins, endotoxins, antigens, lipopolysaccharides, and biologically produced volatile organic compounds. Many occupants of the WDBs reported multiple-system symptoms. Objective measurements indicated that irritant and allergic characteristics of airborne particles adversely impacted pulmonary function. However, methodological limitations in the past had prohibited the definitive attribution of multiple-system illness to the WDB environment. Our previous reports described studies that attempted to overcome some of those limitations [95,97]. Critical study components included a prospective experimental design in which clinical data were collected at five time points; the use of objective indicators of effect; the interventions of successful therapy; removal from exposure; and re-exposure [95]. The study results indicated that SBS acquired in WDBs might be a form of "biotoxin-associated illness", a term used to indicate that some forms of illness may be caused by exposure to toxins produced by single-cell organisms such as fungi, dinoflagellates, cyanobacteria, diatoms, and bacteria. The multiple-system illness identified in patients previously exposed to aquatic environments inhabited by the suspected toxigenic dinoflagellates, *Pfiesteria* sp., represented a form of biotoxin-associated illness [40,96,103], termed "Possible Estuary Associated Syndrome" (PEAS) by the Centers for Disease Control and Prevention [23]. The current study attempted to confirm the previous observations and further characterize the physiological and biochemical characteristics of SBS acquired in WDBs.

Our previous reports described the results from SBS studies that used a powerful ABB′AB study design that allow participants to serve as their own controls. This design provided the advantage that the participants' environmental and genetic characteristics remained constant as they were monitored over time and experimental interventions were enacted. The interventions were the initiation of successful therapy, exposure avoidance, re-exposure, and a second round of therapy. The results showed a spectrum of multiple-system symptoms that were acquired by patients following chronic exposure to WDBs. High symptom prevalence was accompanied by large deficits in visual contrast sensitivity [95,97], an indicator of neurologic function in the visual system [13,48–52,91,103]. Most patients also reported upper airway symptoms, but pulmonary function tests indicated that few patients had obstructive or restrictive lung function. Elevated levels of leptin and decreased levels of alpha melanocyte stimulating hormone (MSH) indicated that the illness likely involved pathology in the hypothalamus. Increased levels of leptin indicated probable damage to leptin receptors in the hypothalamus [44,62,117] that could result from a toxin-triggered increase in the release of pro-inflammatory cytokines by adipose cells [60]. Inability of the damaged leptin receptors to be stimulated by leptin would result in overproduction of leptin [70] and reduced secretion of MSH [43]. MSH deficiency causes abnormal function in the pituitary gland [8], gastrointestinal system [26], and immunologic system [66,67], and are associated with chronic pain [57] and sleep disturbance [18]. Participants received 2 weeks of treatment with cholestyramine (CSM). Cholestyramine is a non-absorbable polymer that removes a variety of toxins from bile through anion-exchange binding, thereby causing toxin elimination rather than reabsorption during enterohepatic recirculation [5,9,15,16,22,25,28,29,54,58,59,64,74,75,86,87,100,111]. CSM therapy resulted in a dramatic decrease in symptom prevalence concomitant with normalization of VCS [95,97]. Group-mean leptin levels were decreased and MSH levels were increased following CSM therapy [95,97]. Health status continued to show marked improvement following CSM therapy while the study participants avoided re-exposure to the WDBs. However, all participants relapsed within 7 days of re-exposure to the WDBs. Readministration of CSM again resulted in a highly significant improvement in the health status indicators. The positive response to toxin-elimination therapy, the maintenance of good health without re-exposure to the WDBs, and the relapse with re-

exposure strongly supported the hypothesis that illness was causally associated with exposure to the WDBs.

The current study again used a prospective experimental design with clinical assessments occurring at five points in time to test the general study hypothesis that SBS is associated with exposure to WDBs. Evaluations occurred immediately after the screening of potential study participants; after 2 weeks of CSM therapy; after the avoidance of exposure to WDBs; following re-exposure to the WDBs; and after a second course of CSM therapy. Symptom prevalence, VCS, pulmonary function, and leptin and MSH concentrations were measured in an attempt to confirm the previously observed results. Levels of immunoglobulin E (IgE), matrix metalloproteinase 9 (MMP9), and vascular endothelial growth factor (VEGF) were measured to help further characterize underlying pathology. A double-blind, placebo-controlled clinical trial was conducted in 13 of the 26 study participants at the onset of the time series-study to assess the efficacy of CSM therapy on health status relative to placebo treatment. Following baseline assessments, the clinical trial participants were randomly assigned to either receive 2 weeks of placebo therapy prior to receiving 2 weeks of CSM therapy or to immediately receive 2 weeks of CSM therapy.

2. Methods

2.1. A priori study hypotheses

2.1.1. General hypothesis

SBS is associated with exposure to WDBs.

2.1.2. Confirmatory hypotheses

2.1.3. Time series study

1.a. Group-mean scores for (i) number of symptoms; (ii) leptin; and (iii) MMP9 show statistically significant decreases between Time Points 1–2, 1–5, and 4–5.

1.b. Group-mean scores for VCS show a statistically significant increase between Time Points 1–2; 1–5; and 4–5.

1.c. The number of participants with abnormal VEGF scores shows a statistically significant decrease between Time Points 1–5.

1.d. Group-mean scores for pulmonary function do not differ significantly between Time Points 1–5.

1.e. The level of MSH is below the normal range at Time Point 1 in >90% of participants.

1.f. The level of IgE is within the normal range at Time Point 1 in >90% of participants.

2.1.4. Double-blind, placebo-controlled, clinical trial

2.a. Relative to initial evaluation (baseline) scores, group-mean scores for number of symptoms and VCS in study participants randomly assigned to the placebo first cohort show no statistically significant improvement after 2 weeks of placebo treatment.

2.b. Group-mean scores for number of symptoms and VCS in participants assigned to the CSM first cohort show statistically significant improvement after 2 weeks of therapy.

2.c. Relative to baseline scores and scores following placebo treatment, group-mean scores for number of symptoms and VCS in participants assigned to the placebo first cohort show statistically significant improvement after 2 weeks of CSM therapy.

2.2. Study participant recruitment and screening

2.2.1. Time series study

Consecutive patients seeking medical attention at a private clinic were screened for possible inclusion in a study of SBS previously approved by an authorized internal review board, Copernicus IRB, Cary, NC. The criteria for possible SBS diagnosis, derived from criteria defined by the Centers for Disease Control and Prevention for diagnosis of PEAS [23], were

1) Exposure potential–chronic and current exposure to the indoor environment of a building showing evidence of water damage and microbial amplification;
2) Multiple system symptoms–symptoms in at least five of the ten organ systems listed in Table 1 that persisted for >2 weeks; and
3) Absence of confounders–the clinician was unable to identify other possible causes of illness using differential diagnosis techniques.

To assess the presence of symptoms and exposure potential, a physician orally administered medical history and exposure questionnaires. Patients reporting water intrusion in the home or workplace, but not both, and visual identification of microbial amplification in the area of water intrusion were eligible for further screening (Table 1). The clinician orally assessed the symptoms of eligible patients by administering a questionnaire in a standard manner with complete recording of all symptoms at each visit (Table 2). Symptoms were categorized into organ systems, with the exception of headache and skin sensitivity. Because headache and skin sensitivity could potentially arise from abnormalities in multiple organ systems (i.e., sinus congestion could cause headache, as could musculoskeletal problems), headache and skin sensitivity were regarded as unique, multifactorial symptoms. Patients meeting criterion 2 above underwent further screening to assess other possible causes of illness. Each potential study participant received a physical examination and blood draw. Blood analyses included a complete blood count and comprehensive metabolic profile. Medical history and additional questionnaires were also used to assess potentially confounding factors, including serious ongoing illness or neurologic disease, alcoholism, occupational or avocational exposure to solvents, petroleum products, metal fumes, or pesticides, previous diagnoses of a PEAS-like illness, Lyme disease, *ciguatera* seafood poisoning, chronic soft-tissue injury, and other medical, environmental, and lifestyle factors. Female participants were excluded from study participation if they were pregnant or might become pregnant during the study, or were nursing a child. Differential diagnosis techniques were used to determine whether or not a cause of illness other than

Table 1
Study participants' characteristics and exposures

Participant number	Age (years)	Gender	Ethnicity	Building type	Predominant fungi	Illness duration (months)	Previous diagnoses	Smoke
1	46	F	C	Occupation	Asp, Pen	12	DD, DJD, TS	P
2	48	F	C	Occupation	Stachy, Asp	2	As, Dp	N
3	65	F	C	Occupation	Asp, Pen, Acre, Tricho	24	As, HBP, NA	N
4	51	F	C	Occupation	Chaet, Asp	3	As, CTS	N
5	65	M	AA	Occupation	Asp, Pen, Acre, Tricho	24	COPD, Dp	N
6	64	F	C	Residence	Asp, Pen, Chaet, Monod, Stachy Visible	3	DJD, HBP, LS	N
7	39	F	C	Occupation	Colonies	6	SA	N
8	43	F	AA	Occupation	Stachy, Asp	24	BC	N
9	41	F	AA	Occupation	Acre, Alten	12	Dp, NA	N
10	51	F	C	Occupation	Stachy	3	As, HBP	N
11	58	F	C	Occupation	Stachy	4	HBP, LD	N
12	32	F	C	Residence	Taeni, Monod	12	NA, Tourette's syndrome	Y
13	61	M	C	Residence	Asp, Pen, Tricho	24	None	N
14	57	F	C	Residence	Asp, Pen, Tricho	24	None	N
15	51	F	C	Occupation	Acre, Alten	12	CPS, Dp, Fm	N
16	58	M	C	Occupation	Stachy, Asp	3	ADHD, Ax, Dp	N
17	52	F	C	Occupation	Asp, Pen	12	DP, NA	N
18	38	F	C	Residence	Asp, Pen	24	Reflux, TMJP	N
19	44	M	C	Residence	Stachy, Chaet, Asp	12	Hair loss, NA, Reflux	N
20	44	F	C	Occupation	Pen, Stachy, Asp, Acre	6	ADHD, Ro	N
21	60	F	C	Occupation	Asp, Pen	2	Raynauds, reflux, vertigo	N
22	47	F	C	Occupation	Stachy	24	Ax, DD, DJD	N
23	52	F	C	Occupation	Asp, Pen	6	As, NA	Y
24	54	F	C	Occupation	Visible colonies	8	Dp, NA	N
25	51	F	C	Occupation	Acre	12	Dp, hypothyroid, migraine	Y
26	34	F	C	Occupation	Acre	6	Migraine, NA, reflux	N
Mean (S.E.M.)	50.2 (1.8)	–	–	–	–	10.8 (1.6)	–	–

M=male, F=female, C=Caucasian, AA=African American, Y=yes, N=no, P=previously smoked, S.E.M.=standard error of the mean.
Acre=*Acremonium*, Alt=*Alternaria*, Asp=*Aspergillus*, Chaet=*Chaetomium*, Monod=*Monodictys*, Pen=*Penicillium*, Stachy=*Stachybotrys*, Taeni=*Taeniolella*, Tricho=*Trichoderma*.
ADHD=attention deficit–hyperactivity disorder, As=asthma, Ax=anxiety, BC=breast cancer, COPD=chronic, obstructive pulmonary disease, CPS=chronic pain syndrome, CTS=carpal tunnel syndrome, DD=demyelinating disease, DJD=degenerative joint disease, Dp=depression, Fm=fibromyalgia, HBP=high blood pressure, LD=lyme disease, LS=lumbar spine, NA=nasal allergy, SA=sleep apnea, Ro=rosacea, TMJP=temporo-mandibular joint pain, TS=thoracic spine.

SBS could be identified. No patients involved in litigation concerning WDBs were offered study participation, and patients were not remunerated for study participation. The first 30 patients of age 18 years or older who met all study inclusion criteria were invited to participate in the study. Voluntary informed consent forms were signed by 28 study participants, whereas two patients chose to receive treatment outside of the study. Two participants were subsequently excluded from the study due to inability to participate in all five study time points, resulting in 26 participants that completed the study (Table 1).

Study participants were requested to collect fungal samples from their water-damaged building areas for laboratory analyses using a protocol provided by P&K Microbiology, Inc. (Cherry Hill, NJ). Bulk or tape lift samples were collected from 24 of the 26 buildings and shipped to P&K Microbiology for fungal analyses. Qualitative microscopic analyses identified one or more genera of fungi colonizing water-damaged surfaces in each building (Table 1). Analyses of mycotoxins, bacteria, endotoxins, and antigens were not undertaken. Photographic evidence of microbial amplification also was provided for each of the buildings.

2.3. Double-blind, placebo-controlled, clinical trial

14 patients who met all of the study inclusion criteria described above volunteered to participate in double-blind, placebo-controlled, clinical trial to assess the efficacy of CSM therapy before continuing with an open-label trial of CSM. Each participant signed a voluntary consent form approved by Copernicus IRB. One participant was subsequently excluded from the study due to inability to complete the study due to schedule conflicts, resulting in 13 participants with complete data sets. No patients who previously participated in SBS studies or clinical trials were included in the clinical trial or time-series study.

2.4. Study design

2.4.1. Time series study

The study used an ABB′AB design that included five time points for medical assessments and three types of interventions. Interventions were CSM therapy, exposure avoidance, and re-exposure. In addition to the symptoms questions, physical examinations, and blood draws administered during screening,

Table 2
Percent of study participants ($n=26$) with symptoms by organ system and time points (T)

Symptom	Organ system	T1	T2	T3	T4	T5
Fatigue	General	92	46	35	100	35
Weakness		58	8	4	58	4
Aches	Musculoskeletal	92	12	8	81	15
Cramps		46	4	0	42	0
Unusual pain		54	0	0	23	0
Ice pick pain		65	4	4	27	0
Lightning bolt pain		19	0	0	0	0
Joint pain		77	27	19	85	35
Morning stiffness		42	0	0	35	0
Headache	Multifactorial;	73	31	19	81	31
Skin sensitivity	unique	8	0	0	0	0
Light sensitivity	Eye	77	8	4	69	0
Red eyes		58	0	0	50	0
Blurred vision		54	4	0	46	4
Tearing		50	0	0	27	12
Sinus	Sinus	85	12	8	69	8
Cough		69	8	19	46	15
Shortness of breath		77	15	12	88	31
Abdominal pain	Gastrointestinal	31	0	0	15	4
Diarrhea		31	4	0	15	0
Numbness	Neurologic	54	8	4	35	0
Tingling		62	8	4	50	4
Metallic taste		38	12	8	15	8
Vertigo		46	15	8	38	8
Memory	Central nervous	85	31	19	65	19
Focus/concentration	system	85	8	12	54	8
Confusion		62	4	0	35	8
Decreased assimilation of new knowledge		69	4	0	31	0
Decreased word finding ability		65	4	0	38	8
Disorientation		19	0	0	0	0
Excessive thirst	Antidiuretic	69	8	0	50	4
Frequent urination	hormone	62	0	0	38	4
Static/shocks		50	0	0	27	4
Night sweats	Hypothalamic	69	27	15	54	4
Mood swings		73	15	4	62	0
Temperature regulation		54	15	0	38	0
Appetite swings		69	12	4	42	0

T1 Baseline.
T2 After first-use cholestyramine.
T3 After cessation of cholestyramine without exposure to WDB.
T4 After cessation of cholestyramine with exposure to WDB.
T5 After repeat treatment with cholestyramine.

study participants underwent visual and pulmonary function testing and additional blood draws during study Time Point 1. The symptoms questions, physical examination, vision testing, and blood draws were repeated at each of the time points with the exception that blood was not drawn during Time Point 3. Pulmonary function measurements were repeated only at Time Point 5. The study interventions were initiated immediately after medical assessments at each of the five time points, and scheduled as

Time Point 1–began CSM therapy for 2 weeks;
Time Point 2–ended CSM therapy and began avoidance of the WDBs for 7 days;
Time Point 3–returned to the WDBs without CSM therapy for 3 days;
Time Point 4–again began CSM therapy for 2 weeks;
Time Point 5–ended CSM therapy.

CSM therapy was prescribed by the physician and administered by the study participants. The dose schedule was 9 g of CSM dissolved in 6 oz. of apple juice or water administered orally, 4 times per day on an empty stomach. Participants were questioned concerning compliance with therapy at Time Point 2–5 medical assessments. All participants reported taking the prescribed dose of CSM 3–4 times per day and were judged as compliant. All study participants reported compliance with WDB avoidance between Time Points 2 and 3, and with re-occupancy of a WDB between Time Points 3 and 4. Participants were advised at the end of the study to initiate prophylactic CSM therapy if they chose to return to the WDB environment prior to successful building remediation.

2.5. Double-blind, placebo-controlled, clinical trial

13 participants were randomly assigned to first receive CSM or placebo treatment for 2 weeks. A clinical staff member prepared 56 single-dose packets (4 times/day for 14 days) of CSM or placebo, labeled “A” or “B”, for each study participant. Neither the study physician nor the study participants knew which packets contained medication or placebo. Following the clinical evaluations after 2 weeks of treatment, the participants that had received the packets containing placebo received 2 weeks of CSM therapy. VCS and symptoms were assessed immediately prior to the initiation of treatment, after 2 weeks of treatment, and for those in the placebo first group, after an additional 2 weeks during which CSM therapy was assigned. Participants were questioned concerning compliance with treatment during each clinical evaluations session. All participants reported taking the prescribed dose from packets “A” or “B” 3–4 times per day, and were judged as compliant.

2.6. Blood analyses–time series study

Blood analyses were performed by LabCorp, Inc., and Quest Diagnostics, Inc., each of which is a CLIA-approved, high-complexity laboratory facility (Table 3).

IgE was measured by LabCorp, Inc. (which reported a normal range of 0–165 IU/ml serum) only at study Time Point 1. Because the confirmatory hypothesis was that IgE level would be normal in most study participants at Time Point 1, it was determined that IgE level would not be measured at other study time points if the hypothesis was confirmed.

MSH was measured by LabCorp, Inc. (which reported a normal range of 35–81 pg/ml serum) only at study Time Point 1. We previously reported that MSH was abnormally low in almost all SBS cases prior to CSM therapy, and that although there was a statistically significant increase in group-mean MSH levels following therapy, few cases had returned to the normal range after 2 weeks of therapy [95].

Normal ranges for leptin in men, 2–13 ng/ml serum, and in women, 4–25 ng/ml serum, unadjusted for body mass, were

Table 3
Study participant biomarker characteristics across clinical examination time points

Time point	Mean IgE (IU/ml)/S.E.M. (#above norm)	Mean MSH (pg/ml)/S.E.M. (#below norm)	Mean FVC/S.E.M. (#below norm)	Mean FEV1/FVC × 100/S.E.M. (#below norm)	Mean leptin (ng/ml)/S.E.M. (#above norm)	Mean MMP9 (ng/ml)/S.E.M. (#above norm)	Mean VEGF (pg/ml)/S.E.M. (#below, norm, above)
1	56.4/78.1(1)	16.1/7.7(25)	88.6/2.4(5)	103.9/3.0 (2)	27.1/4.3 (13)	563/48.5 (22)	58.6/10.1 (9,12, 5)
2	NA	NA	NA	NA	25.1/3.9 (11)	379/48.4 (15)	56.7/11.1 (4, 14, 2)
4	NA	NA	NA	NA	30.4/4.7 (14)	571/56.8 (18)	66.5/15.8 (5, 12, 4)
5	NA	NA	85.5/2.3(6)	106.8/2.9 (1)	23.4/3.8 (10)	308/32.0 (10)	56.6/6.6 (3, 17, 2)

S.E.M. = standard error of the mean, NA = not available. Normal: FVC > 80, FEV1/FVC × 100 > 80.
Normal ranges: IgE (LabCorp, Inc.) = 0–165 IU/ml; MSH (LabCorp, Inc.) = 35–81 pg/ml; Leptin (LabCorp, Inc.), Men = 2–13 ng/ml, Women = 4–25 ng/ml; MMP9 (Esoteric, Inc.) = 0–332 ng/ml; VEGF (Quest Diagnostics, Inc.) = 31–86 pg/ml.

reported by LabCorp, Inc. Leptin levels were measured at study Time Points 1, 2, 4, and 5.

MMP9 levels were measured by Quest Diagnostics, Inc. (which reported a normal range of 0–332 ng/ml serum), at study Time Points 1, 2, 4, and 5.

VEGF levels were measured by Quest Diagnostics, Inc. (which reported a normal range of 31–86 ng/ml serum) at study Time Points 1, 2, 4, and 5.

2.7. Pulmonary function testing—time series study

Pulmonary function was assessed in all patients using a Micro Medical spirometer. Forced expiratory volume in 1 s (FEV-1) and forced vital capacity (FVC) were measured. Pulmonary function in each participant was categorized according to the criteria

Normal: FEV-1/FVC × 100 > 80; FVC > 80;
Obstructive (e.g., asthma): FEV-1/FVC × 100 < 80; FVC > 80;
Restrictive (e.g., hypersensitivity pneumonitis): FEV-1/FVC × 100 > 80; FVC < 80;
Obstructive and Restrictive: FEV-1/FVC × 100 < 80; FVC < 80.

2.8. Visual function testing

Near-point visual acuity and near-point VCS were measured using modified, forced-choice procedures previously described in detail [52,95,97]. Briefly, the acuity (MIS Pocket Vision Guide, 1997 MIS, Inc.) and VCS (Functional Acuity Contrast Test, F.A.C.T. 101; Stereo Optical Co., Chicago, IL, a Gerber-Coburn Co.) tests were administered monocularly to each eye under standard day-light spectrum illumination fluorescent bulbs with normal office background illumination. Photometric measurements indicated a luminance of greater than 70 ft L on the test card surfaces. All participants who used corrective lenses for near-point viewing wore them during vision testing. The contrast sensitivity test card contained a matrix (5 × 9) of circles filled with sinusoidal gratings (dark and light bars). Spatial frequency (1.5, 3, 6, 12 and 18 cycles/degree of visual arc) increased from top to bottom, and contrast decreased from left to right in steps of approximately 0.15 log units. The grating bars were oriented either vertically, or tilted 15° to the left or right. As the investigator called out each circle from left to right, row by row, subjects responded by saying either vertical, left, right or blank. Participants were encouraged to name an orientation if they had any indication that the bars could be seen. Participants were given the option to point in the direction to which the top of the grating was tilted if they felt any difficulty in verbalizing the orientation; none needed this assistance. The contrast sensitivity score for each row (spatial frequency) was recorded as the contrast of the last test patch correctly identified on that row following verification by repeated testing of that patch and the subsequent patch. The procedure was repeated for each row in descending order.

The *a priori* criterion for analysis of VCS data, that the eye had a Snellen Distance Equivalent visual acuity sore of 20:50 or better, was met by both eyes in 23 of the study participants. At least one eye of each participant met the acuity criterion. This criterion avoided confounding of VCS scores as indicators of neurologic function by excessive optical-refraction error. The units of analysis of group-mean VCS scores were the mean scores of the participants' two eyes at each spatial frequency except for three incidences where one eye did not meet the acuity criterion.

Assessments of VCS scores in individuals were based on criteria applied to the mid-three spatial frequencies (rows B, C, and D, 3.0, 6.0 and 12.0 cycles/degree of visual arc, respectively). Criteria for normal VCS were

(A) If VCS at row B = 160 (stimulus ID 9), then VCS at row C > 128 (stimulus ID 8) and row D > 43 (stimulus ID 6);
(B) If VCS at row B < 160 (stimulus ID 9), then VCS at row C > 90 (stimulus ID 7) and row D > 43 (stimulus ID 6).

If either eye of a participant failed to meet the criteria for normal VCS, then VCS in that participant was classified as abnormal.

2.9. Statistical analyses

All statistical analyses tested a two-tailed hypothesis of no difference at a significance level of alpha = 0.05. The statistical procedure used to analyze the VCS data was multivariate analysis of variance for repeated measures (MANOVA). The VCS units of analysis were considered to be repeated measures because one unit for each of the five spatial frequencies

was collected from each study participant in the same order at each of the five time points. The five VCS units collected from each participant at one time point were compared to the five VCS units collected at another time point in one analysis. This procedure was repeated for all 10 possible pairings of data collected in the time series study and the six possible pairings in the clinical trial of the efficacy of CSM therapy. Bonferroni adjustments were made to all *p*-values to reduce the possibility of false-positive results due to the multiplicity of comparisons.

Three hypotheses were tested in each of the MANOVA analyses of the VCS data. The first null hypothesis was that there was no difference between the data collected at the two time points being compared. The analysis essentially averaged the five group-mean VCS units collected at each of two time points, and determined whether or not there was a statistically significant difference between the two time points. The second null hypothesis was that there was no difference between the VCS units collected at each of the five spatial frequencies. This analysis averaged the VCS units from both time points at each of the five spatial frequencies, and determined whether or not there was a statistically significant difference among the five averages. This hypothesis should always be rejected because VCS scores are known to vary with spatial frequency. Rejection of this hypothesis essentially confirmed the validity of the VCS procedure, and the results are not reported when the hypothesis was rejected. The third hypothesis was that there was no interaction between study time points and the VC scores at each of the five spatial frequencies. This test essentially determined whether or not the VCS spatial-frequency profiles from two study time points were parallel. Rejection of the hypothesis indicated that there was a spatial frequency-dependent change in sensitivity between time points.

The same statistical approach was used to assess differences between time points in symptom, pulmonary function (FVC and FEV1/FVC), leptin, and MMP9 levels. Each of those variables, when measured, was measured only once at each time point. In this case, the MANOVA analyses reduced to paired *t*-tests that were done for comparisons at each possible pair of times. Because prior clinical observations indicated that SBS patients could have abnormally low or high VEGF levels prior to CSM therapy, participants' VEGF levels were categorized as normal or abnormal at each time point when measured. Chi-square tests were used to assess the statistical significance of differences in the number of normal and abnormal levels between time points. Only variable pairs with data at both times were used in the comparisons. Bonferroni adjustments were made to the *p*-values to control for the multiplicity of comparisons.

3. Results

3.1. Time series study

3.1.1. Building descriptions

All 26 study buildings had visible evidence of water damage and microbial amplification (Table 1). Plumbing leaks, water intrusion in basements, in or around the roof and behind window and door casements, and flooding were the primary causes of water damage. Qualitative laboratory analyses of tape lift or bulk samples revealed one or more sites of fungal colonies in each of the 24 buildings sampled. Analysis of the samples indicated the fungal genera of *Aspergillus* sp. (16 samples), *Penicillium* sp. (10 samples), and *Stachybotrys chartarum* (9 samples) predominated (Table 1).

3.2. Participant demographics

Twenty study participants received occupational exposure to WDBs, whereas six participants resided in WDBs (Table 1). Participants ranged in age from 32 to 65 years, 4 were male and 22 were female, 3 were of African American ethnicity and 23 were Caucasian (Table 1). Illness duration ranged from 3 to 24 months (Table 1). Each participant was exposed to the same WDB throughout the illness period, including when the study commenced. No repairs, remediation or other changes were known to have occurred in any of the building structures or ventilation systems during the study. The participants previously received a variety of diagnoses during their current illnesses from physicians unaffiliated with the study (Table 1). Three participants regularly smoked cigarettes, and one participant had previously smoked on a regular basis (Table 1). Each participant reported little or no relief from symptoms when away from the WDB while on business travel or vacation. Twenty-six of 28 participants completed the study, each within a 38-day time period.

3.3. Time Point 1 assessment

3.3.1. Symptoms

The 26 participants who completed the study averaged 22.8 (S.E.M. = 1.2) symptoms out of 37 at Time Point 1 (Fig. 1A). All participants had symptoms from at least five of the ten organ systems assessed, indicating multiple-system illness. The number of symptoms ranged from 14 to 32. The percentages of participants that reported each symptom are shown in Table 2. Over 60% of the study participants reported fatigue, muscle aches, ice pick pain, joint pain, headache, light sensitivity, sinus congestion, cough, shortness of breath, tingling in extremities, memory loss, difficulty with concentration, confusion, difficulty with the assimilation of new knowledge and word finding ability, excessive thirst, frequent urination, night sweats, mood swings, and appetite swings at Time Point 1.

3.3.2. VCS

Although group-mean Snellen visual-acuity scores were normal (Left eye = 20:23.2; Right eye = 20:22.7), group-mean VCS scores (Fig. 1B) were sharply reduced relative to the previously published control values [95]. The VCS deficits were largest at the mid-to-higher spatial frequencies. VCS was classified as abnormal in all 26 participants at Time Point 1.

3.3.3. Pulmonary function

Test results indicated normal function in 19 participants, a restrictive condition in five participants (including two current smokers), and an obstructive condition in two participants at

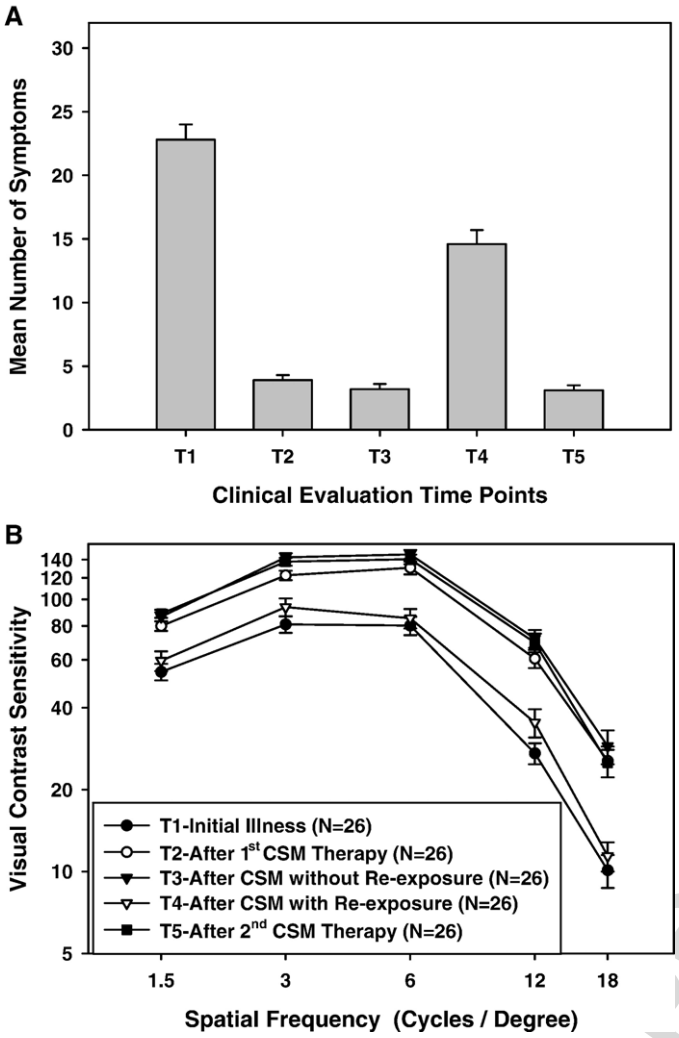

Fig. 1. (A) Displays the group-mean number of symptoms at each of the 5 time points in the time series study. The curves in (B) display the visual contrast sensitivity profiles across spatial frequencies at each of the 5 time points. Error bars indicate the standard errors of the mean. Symptom and VCS scores moved in synchrony during the study in response to CSM therapy and exposure to the indoor air of water-damaged buildings.

Time Point 1 (Table 3). No participants had both a restrictive and obstructive condition.

The results from laboratory measurements of biochemical levels are shown in Table 3.

3.3.4. IgE

Levels were within the normal range of 0–165 IU/ml in 25 participants. IgE was 354 IU/ml in one participant who had normal lung function.

3.3.5. MSH

Levels were below the normal range of 35–81 pg/ml in 25 participants; MSH was 37 pg/ml in one participant.

3.3.6. Leptin

Levels were within the normal range in 50% of the participants, whereas levels in 1 male and 12 females exceeded the normal limit.

3.3.7. MMP9

Levels exceeded the normal limit of 332 ng/ml in 22 participants. MMP9 levels were 138,179, 224, and 247 in the other four participants.

3.3.8. VEGF

Levels were normal in 12 participants, below the normal limit of 31 pg/ml in 9 participants, and above the normal limit of 86 pg/ml in 5 participants.

The application of differential diagnosis techniques to the data from the questionnaires on medical history and confounding factors, the physical examination, vision and pulmonary function testing, and the laboratory analyses indicated no possible cause of illness other than chronic exposure to the WDBs in any participant.

3.4. Time Point 2 assessment

All participants showed marked improvement at Time Point 2 following 2 weeks of CSM therapy.

3.4.1. Symptoms

The group-mean number of symptoms decreased to 3.9 (Fig. 1A), a statistically significance difference from Time Point 1 [$p<0.001$, all p-values are Bonferroni corrected]. The prevalence of each symptom decreased, although more than 25% of the participants continued to experience fatigue, joint pain, headache, memory difficulty, and night sweats (Table 2).

3.4.2. VCS

Scores at the mid-spatial frequency increased by almost 65% following CSM therapy. The robust recovery in mean VCS (Fig. 1B) between Time Points 1 and 2 was statistically significant [$p<0.001$]. The VCS spatial frequency profile normalized, showing peak sensitivity at 6 cpd, resulting in a statistically significant spatial frequency by Time Point 1 vs 2 interaction term [$p<0.001$]. VCS scores returned to normal levels in 21 of 26 participants.

3.4.3. Leptin

Levels decreased in 14 participants at Time Point 2, but only 2 of the 13 participants with abnormally high levels at Time Point 1 had returned to the normal range. Group-mean leptin concentration decreased (Table 3), although insignificantly [$p=0.229$].

3.4.4. MMP9

The decrease in group-mean MMP9 concentration was statistically significant [$p<0.01$]. MMP9 levels decreased in 21 of the 22 participants who had abnormally high levels at Time Point 1, although levels remained above the upper limit of the normal range in 15 participants (Table 3).

3.4.5. VEGF

Of the nine participants that had abnormally low levels of VEGF at Time Point 1, four increased to the normal range, three remained abnormally low, and data were missing for two.

VEGF levels decreased to the normal range in 3 of 5 participants who had abnormally high levels at Time Point 1. One participant remained above the normal range, and data were missing for the other participant. Of the 12 participants that had normal VEGF levels at Time Point 1, one increased and one decreased to an abnormal level, and data were missing for three (Table 3). The increase in the number of participants with normal VEGF levels between Time Points 1 and 2 was not statistically significant.

3.5. Time Point 3 assessment

Recovery continued at Time Point 3 as participants avoided exposure while no longer on CSM therapy.

3.5.1. Symptoms

The group-mean number of symptoms was 3.2 (Fig. 1A), a level significantly lower than at Time Point 1 [$p<0.001$], but not significantly lower than at Time Point 2 [$p<0.115$]. Symptom prevalence remained stable or declined further for all but two symptoms (Table 2).

3.5.2. VCS

Group-mean VCS scores showed further improvement (Fig. 1B) to a level significantly higher than at Time Points 1 [$p<0.001$] and 2 [$p=0.045$]. The mid-spatial frequency VCS score was 82% greater than that measured at Time Point 1. The time point by spatial frequency interaction term did not show a statistically significant difference between Time Points 2 and 3 [$p=0.499$], reflecting the maintenance of peak sensitivity at 6 cpd (Fig. 1B). VCS was classified as abnormal in only one participant.

Biochemical levels were not measured at Time Point 3.

3.6. Time Point 4 Assessment.

All participants returned to the WDBs for 3 days without CSM therapy following the assessment at Time Point 3. All participants reported relapse when assessed at Time Point 4.

3.6.1. Symptoms

The group-mean number of symptoms increased to 14.6 (Fig. 1A), a level significantly higher than that at Time Points 2 [$p<0.001$] and 3 [$p<0.001$], but significantly lower than that at Time Point 1 [$p<0.001$]. The prevalence of all but three symptoms was greater at Time Point 4 than at Time Point 3 (Table 3).

3.6.2. VCS

Group-mean VCS scores (Fig. 1B) declined to a level significantly below that measured at Time Points 2 [$p<0.001$] and 3 [$p<0.001$], but was similar to that observed at Time Point 1 [$p=0.531$]. The VCS score at mid-spatial frequency declined by 42%. The statistically significant difference in the spatial frequency by Time Point 3 vs 4 interaction term [$p=0.015$] reflected the shift in peak sensitivity from the mid-spatial frequency of 6 cpd to 3 cpd at Time Point 4. Only three participants demonstrated normal VCS at Time Point 4.

3.6.3. Leptin

Group-mean leptin concentration (Table 3) was significantly higher than at Time Point 2 [$p=0.050$]. Leptin levels at Time Point 4 showed an increase in 16 participants relative to Time Point 2, and were abnormally high in 14 participants. Leptin data were missing for one participant who had abnormally high levels at Time Points 1 and 2.

3.6.4. MMP9

The increase in group-mean MMP9 concentration between Time Points 2 and 4 (Table 3) was statistically significant [$p<0.01$]. MMP9 levels increased in 22 participants and declined in two participants. MMP9 data were missing for two participants who had abnormally high levels at Time Point 1 but normal levels at Time Point 2. Of the 22 participants that had abnormally high MMP9 levels at Time Point 1, only three had normal levels at Time Point 4.

3.6.5. VEGF

Levels were abnormally low in 5 participants, abnormally high in 4 participants, and normal in 12 participants. Data were missing for 5 participants, 2 of whom were abnormally low at Time Point 1. The change in the proportion of participants with normal VEGF levels between Time Points 2 and 4 (Table 3) was not statistically significant.

3.7. Time point 5 assessment

Following relapse during the re-exposure phase of the study, participants again underwent CSM therapy for 2 weeks. Health assessments at Time Point 5 indicated substantial improvement in all participants.

3.7.1. Symptoms

The group-mean number of symptoms reported decreased from 14.6 to 3.1 (Fig. 1A), a level significantly lower than that at Time Point 4 [$p<0.001$], but similar to that reported at Time Points 2 [$p>0.999$] and 3 [$p>0.999$]. The prevalence of all symptoms decreased between Time Points 4 and 5 other than those that were 0 at Time Point 4 (Table 2).

3.7.2. VCS

Scores showed a similar pattern of improvement. Group-mean VCS scores at Time Point 5 (Fig. 1A) were significantly higher than at Time Point 4 [$p<0.001$], but similar to that observed at Time Points 2 [$p=0.143$] and 3 [$p=>0.999$]. VCS increased by 69% at the mid-spatial frequency between Time Points 4 and 5. The significant difference in the spatial frequency by Time Point 4 vs 5 interaction term [$p<0.001$] indicated that the shape of the spatial frequency profile was altered. A restoration of peak sensitivity at the mid-spatial frequency of 6 cpd is apparent at Time Point 5 (Fig. 1A). VCS was classified as normal in 21 of 26 participants, as was the case at Time Point 2. Group-mean Snellen visual-acuity scores (Time

Point 5: Left eye=20:22.0; Right eye=20:23.2) did not differ significantly in any pair of study time point comparisons.

3.7.3. Pulmonary function

There was little change in pulmonary function test results between Time Points 1 and 5 (Table 3). Neither the changes in group-mean FVC nor FEV-1/FVC×100 were statistically significant [$p=0.184$ and 0.198, respectively]. However, individual changes between Time Points 1 and 5 included the conversions of a restrictive condition to normal function (smoker), normal function to a restrictive condition (non-smoker), and an obstructive condition to a restrictive condition (non-smoker).

3.7.4. Leptin

The decrease in group-mean leptin levels between Time Points 4 and 5 (Table 3) was statistically significant [$p<0.01$], as was the decrease between Time Points 1 and 5 [$p=0.012$]. Leptin levels decreased in 22 participants, but remained abnormally high in 10 participants at Time Point 5.

3.7.5. MMP9

The group-mean decrease in MMP9 between Time Points 4 and 5 was statistically significant [$p<0.01$], as was the decrease between Time Points 1 and 5 [$p<0.01$]. MMP9 levels decreased in 22 participants between Time Points 4 and 5, although 10 participants remained at abnormally high levels (data missing for three participants, two of whom had high levels a Time Point 4).

3.7.6. VEGF

The proportion of participants that had normal VEGF levels at Time Point 5 (Table 3) was significantly greater than at Time Point 1 [$p=0.03$]. Of the five participants that had low VEGF levels at Time Point 4, four were in the normal range at Time Point 5. All four participants that had high VEGF levels at Time Point 4 were in the normal range at Time Point 5. However, two participants had low VEGF levels at Time Point 1, normal levels at Time Point 4, and low levels at Time Point 5. Of the two participants that had high VEGF levels at Time Point 5, one had normal levels at Time Points 1 and 4, whereas the other had a low level at Time Point 1 and a normal level at Time Point 4.

3.8. Double-blind, placebo-controlled, clinical trial

A double-blind, placebo-controlled clinical trial compared the efficacy of CSM and placebo treatments on symptoms and VCS in patients diagnosed with SBS.

3.8.1. Symptoms

The group-mean number of symptoms at baseline was 24.7 in the cohort randomly assigned to receive CSM first, and 20.8 in the placebo-first cohort (Fig. 2A). The difference between cohorts in number of symptoms at baseline was not statistically significant [$p=0.324$]. Following 2 weeks of treatment, the group-mean number of symptoms showed a statistically significant decline to 2.86 in the CSM-first cohort [$p<0.001$], but remained elevated at 20.3 [$p=0.688$] in the placebo-first cohort (Fig. 2A). Subsequently, following 2 weeks of CSM therapy, the group-mean number of symptoms in the placebo-first cohort declined from baseline to 4.55 [$p=0.004$ (Fig. 2A).

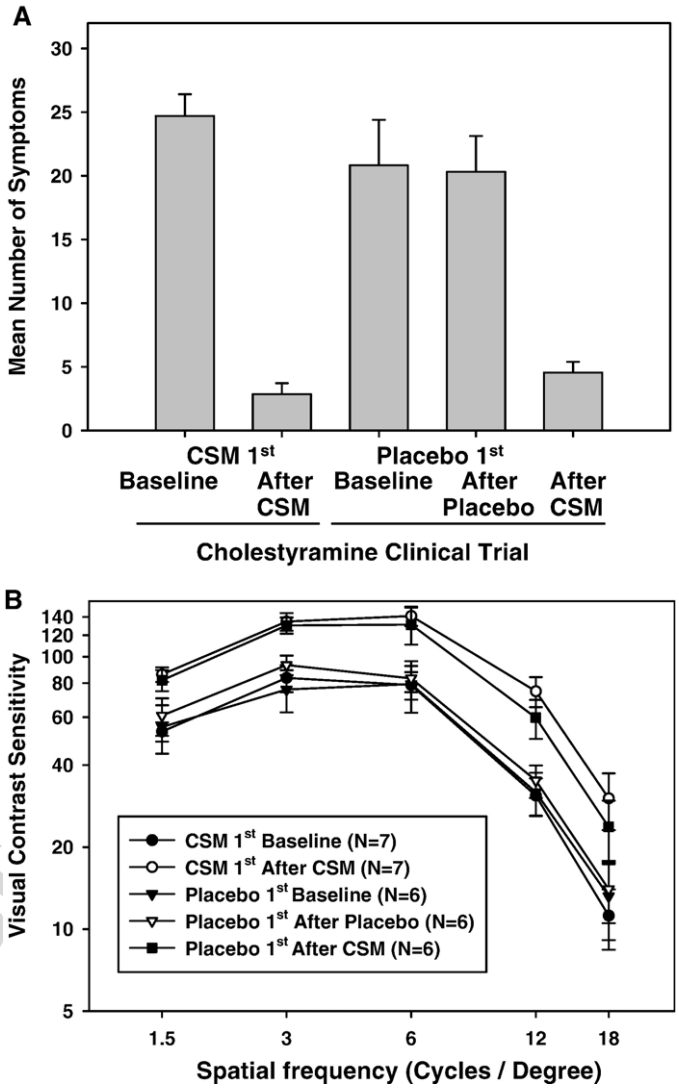

Fig. 2. (A) Displays the group-mean number of symptoms during the double-blinded, placebo-controlled clinical trial on the efficacy of cholestyramine therapy. The curves in (B) display the visual contrast sensitivity profiles across spatial frequencies during the experimental conditions. Error bars indicate the standard errors of the mean. Symptom and VCS scores improved during CSM therapy, but not during placebo therapy.

3.8.2. VCS

At baseline, VCS was markedly depressed relative to previously published control values [95] in both the CSM first and the placebo first cohorts (Fig. 2B). The differences between cohorts in group-mean VCS scores [$p>0.999$] and the group by spatial frequency interaction term [$p>0.999$] was not statistically significant. VCS was classified as abnormal in the seven members of the CSM first cohort and the six members of the placebo first cohort. VCS at the mid-spatial frequency increased by over 75% in the CSM first cohort following 2 weeks of therapy. The difference in mean VCS was statistically significant [$p=0.006$], as was the spatial frequency by

time point interaction term [$p=0.06$]. The shift in peak sensitivity from 3 cpd at baseline to 6 cpd after therapy is apparent in Fig. 2B. VCS was classified as normal in 6 of the 7 CSM first cohort members. Mean VCS in the placebo first cohort did not change significantly [$p>0.999$] following 2 weeks of placebo treatment. However, following 2 weeks of CSM therapy, VCS at the mid-spatial frequency increased by over 65% in the placebo first cohort, a statistically significant increase [$p=0.012$]. VCS remained abnormal following placebo treatment in all six members of the placebo first cohort, but was restored to normal in five of the six members following CSM therapy. There was not a significant change in Snellen visual-acuity scores in any of the statistical comparisons.

4. Discussion

The results from the time series study both confirmed and expanded upon results previously reported. All participants selected for study inclusion presented evidence of exposure potential, a multiple-system illness, and an absence of confounding factors.

- *Symptoms*: The medical assessments conducted at Time Point 1 indicated an average of 23 out of 37 symptoms in multiple organ systems. Below normal concentrations of MSH in 25 of the 26 participants at Time Point 1 (Hypothesis 1.e.) indicated hypothalamic involvement and confirmed previous results [95,97].
- *VCS*: Large and statistically significant increases in VCS between Time Points 1–2, 15, and 45 (Hypothesis 1.b.) indicated improved neurologic function following CSM therapy. Statistically significant decreases in the number of symptoms occurred concomitant with the improvement in VCS (Hypothesis 1.a.), confirming previous results [95,97].
- *Pulmonary function*: The measurements of pulmonary function were not statistically different between Time Points 1–5 (Hypothesis 1.d.), consistent with the IgE indication of lack of an allergic response.
- *IgE*: Normal IgE levels in 25 of the 26 participants at Time Point 1 (Hypothesis 1.f.) indicated that illness was not of atopic origin.
- *Leptin*: The statistically significant decreases in leptin concentrations between Time Points 1–5 and 4–5 (Hypothesis 1.a.), but not 1–2, also confirmed previous results [95] and indicated a possible recovery of function in leptin receptors following CSM therapy.
- *MMP9*: Levels of MMP9 showed statistically significant decreases between Time Points 1–2, 1–5, and 4–5 (Hypothesis 1.a.), indicating a lessening of disease and inflammation.
- *VEGF*: The number of participants with normal levels of VEGF showed a statistically significant improvement between Time Points 1–5 (Hypothesis 1.c.), indicating decreased tissue hypoxia.

These results supported the confirmatory hypotheses, thereby supporting the general hypothesis that SBS is associated with exposure to WDBs.

The results from the double-blind, placebo-controlled clinical trial indicated that cholestyramine therapy is effective at restoring health in SBS patients. Statistically significant improvement was seen in the group-mean number of symptoms and VCS scores following cholestyramine treatment (Hypotheses 2.a. and 2.c.), but not following placebo therapy (Hypotheses 2.b.). These results were consistent with those from a similar clinical trial conducted on PEAS patients [94]. Because the only known beneficial effect of cholestyramine therapy is to greatly enhance the elimination rates of toxins by preventing their reabsorption during enterohepatic recirculation, these results supported the hypothesis that SBS associated with exposure to WDBs is a biotoxin-associated illness.

Occupancy of WDBs that show evidence of microbial amplification presented the opportunity for airborne exposure to a complex mixture of substances including fungi, mycotoxins, bacteria, endotoxins, antigens, lipopolysaccharides, and biologically produced volatile organic compounds [10–12,32,47,78,81,82,92,93,95]. The current study recorded symptoms to describe the involvement of multiple-systems in the illness reported by exposed patients. VCS was measured to obtain objective evidence of alterations in neurologic function. The rapid changes in VCS coinciding with CSM therapy have been reported in multiple studies previously, coinciding with initiation of CSM therapy [48,94–97,100], also associated with reduction of MMP9, and leptin [97]. These findings suggest that improvement of VCS is associated with reduction of levels of pro-inflammatory cytokines by CSM therapy. Serial measurements of velocity of blood flow in capillaries of retina and neural rim of optic nerve head demonstrated by use of the Heidelberg Retinal Flowmeter (data unpublished) confirm improvement of capillary hypoperfusion with CSM therapy, an observation consistent with reduction of levels of pro-inflammatory cytokines [30,34,97,113,114]. The biomarkers of leptin, MMP9, VEGF, and MSH were measured to gain insight into some of the mechanisms active in illness induction. The results indicated a possible cascade of effects that ranged from peripheral induction of pro-inflammatory cytokines and disruption of capillary responses to hypoxia to disruption of the proopiomelanocortin pathway in the hypothalamus and ensuing loss of regulatory control processes. The following model describes modes of action along a pathway leading to biotoxin-associated illness for the purpose of organizing current knowledge into testable hypotheses.

Many of the exposure mixture components found in WDBs have been reported to up-regulate release of pro-inflammatory cytokines, including certain endotoxins [89], lipopolysaccharides [116], fungal fragments [38,39,77,88], and fungal spores and mycotoxins [33,41,42,45,53,55,71,84,85,102,115]. For example, lipopolysaccharides and fungal elements are agonists of Toll 4 receptors on macrophages. Activation of the Toll-like receptors induces synthesis of pro-inflammatory cytokines [39,77,88,112,116]. Stimulation of adipocytes by some of the mixture components also induces the release of large quantities of pro-inflammatory cytokines [63]. MMP9 is a hallmark of elevated pro-inflammatory cytokine levels. MMP9 is one of 24 separate extracellular matrix-degrading endopeptidases secreted during

repair and remodeling processes in disease and inflammation [80]. The production of MMP9 is regulated at the transcriptional level by specific signals initiated by inflammatory responses [80]. Because MMP9 is rarely expressed in healthy tissues, it is a sensitive indicator of a cytokine-induced inflammatory response. The observation of elevated levels of MMP9 at Time Points 1 and 4 in the current study indicated exposure to the WDBs induced an inflammatory response. Additional research is needed to elucidate relationships between exposure to the complex mixture of substances commonly observed in WDBs and the initiation of a pro-inflammatory cytokine response.

Another action of circulating pro-inflammatory cytokines is the stimulation of leptin release from adipocytes [35,62,63,90]. Leptin has two important functions in the biotoxin pathway, triggering macrophage synthesis of additional pro-inflammatory cytokines in a positive feedback loop [39,77,88] and the initiation of negative feedback control on cytokine production through the proopiomelanocortin (POMC) pathway in the ventromedial nucleus of the hypothalamus [1,31,43,68,73,104]. Leptin links neuroendocrine and immune systems by binding to the long isoform of the leptin receptor, which resembles a gp-130 cytokine receptor, thereby stimulating POMC expression and depolarization of POMC-containing neurons [31,44]. POMC is cleaved to produce beta endorphin and MSH. MSH provides regulatory control of the peripheral cytokine response [65,104] and intracerebral inflammation [66,98]. Anti-inflammatory actions of MSH derive from modulation of protein kinase a, p38 kinase, nuclear factor kappa B (NFkB) signaling pathways [14,19,21,56,116], modulation of Toll-like receptor activation by antigens [106], and modulation of T cell responses [76,105–108]. MSH thereby reduces expression of pro-inflammatory cytokines such as tumor necrosis factor alpha, interleukin 1 beta, interleukin 6, interferon gamma [116]. However, high levels of pro-inflammatory cytokines, especially interferons, block expression of the POMC gene by binding to the long isoform of the leptin receptor [101]. The receptor blockage creates leptin resistance, similar to the insulin resistance caused by blockage of the insulin receptor in Type II diabetes [69]. The leptin resistance created by receptor blockage causes reduced activation of the Janus kinase mechanism that activates transcription of the genes for POMC production and subsequent release and cleavage to MSH [37]. Leptin resistance also causes increased leptin production [46,117] and the cascading effect of increased production of pro-inflammatory cytokines [62,67,83]). These responses of leptin and MSH to pro-inflammatory cytokines are consistent with the observations of high leptin and low MSH levels at Time Point 1 in the current study. It is hypothesized that therapeutic administration of MSH [20,65] to SBS patients would dampen the pro-inflammatory cytokine response, reduce leptin concentration, and lessen the number and severity of other signs and symptoms.

Pro-inflammatory cytokines constrict microvasculature and reduce blood flow rates creating hypoperfusion and tissue hypoxia [30,34,97,98,113,114]. Oxygen deprived tissues release hypoxia inducible factor [36,109], an initiator of VEGF transcription [36,99]. The release of VEGF, a promoter of angiogenesis during tissue repair [61,99] is also stimulated by MMP9 [1,7]. Group-mean VEGF levels in the current study were lower following CSM therapy. However, VEGF levels were abnormally high in only approximately one fifth of the study participants at Time Point 1 and abnormally low in approximately one third. Although the mechanism responsible for abnormally low VEGF levels is unclear, VEGF deficiency is implicated in failure of neuroprotection [6]. Over three fourths of the participants had VEGF levels in the normal range at Time Point 5, whereas levels remained abnormally low in three participants and abnormally high in two participants. Additional research is needed to characterize the role of VEGF in the biotoxin pathway, which may involve a biphasic response of initial increases followed by depletion during illness.

The current and previous studies [95,97] were unable to investigate the potential association of SBS with particular microbes, biotoxins, or other components in the complex mixtures observed in the indoor air of WDBs. The composition of indoor air pollutants varies over time in WDBs as organisms compete for survival on water-damaged surfaces [72]. Although long-term air monitoring could partially characterize the airborne mixture, it is generally agreed that not all bioactive components of such mixtures have been identified [95]. Furthermore, few tests have been developed for identifying and quantifying specific mycotoxins, endotoxins, and other mixture components in human tissues, although two trichothecene mycotoxins produced by *Stachybotrys*, satratoxin and roridin, have been identified in serum using an ELISA assay [10,11]. The variety of fungi identified in the current study and the wide variety of mixture components identified in previous studies [95,97] indicated that multiple components may be involved in the etiology of SBS. Animal models have demonstrated synergistic interactions between mixture components in the induction of a pro-inflammatory cytokine response. Mixtures of *Streptomyces californicus* and metabolites from *S. chartarum* and other fungi caused a synergistic release of tumor necrosis factor alpha and interleukin-6 by mouse RAW264.7 macrophages [55]. It is likely, therefore, that many of the mixture components, including fungi, bacteria, mycotoxins, endotoxins, and lipopolysaccharides interact, possibly synergistically, through the common pathway of pro-inflammatory cytokine induction in WDB-associated SBS.

Because of the potential for interactions between mixture components, it is inappropriate to conclude that chronic exposure to the indoor air of WDBs cannot cause toxicity in humans because the dose of a single mixture component is unlikely to reach a toxic level. The position paper by the American College of Occupational and Environmental Medicine stated "that delivery by the inhalation route of a toxic dose of mycotoxins in the indoor environment is highly unlikely at best, even for the hypothetically most vulnerable subpopulations" [2]. This conclusion was based on the observation of pulmonary inflammation in mice following subchronic exposure to a cumulative dose of 2.8×10^5 s. 72 *S. chartarum* spores/kg body weight administered over a 3-week period [79], estimated to correspond to 9.4×10^3 spores/m^3 for infants, and the report that concentration of *S. chartarum* reached only 118 CFU/m^3 in a survey of buildings whose occupants did not report SBS [93].

Not only did this conclusion neglect the potential for interactions between mixture components in the induction of SBS, but also neglected other important considerations.

1) A no-observable adverse effect level (NOAEL) was not identified in the rodent study [79]. Pulmonary inflammation may occur at dosages below 2.8×10^5 spores/kg body weight. Another study of fungal-induced pulmonary inflammation estimated the NOAEL to be $<3.0\times10^4$ spores *S. chartarum*/kg body weight [33].
2) Airborne fungal spores carry only a fraction of the mycotoxins and other biologically active mixture components to which people are exposed in WDBs. The concentration of small fungal fragments carrying mycotoxins, antigens, and other biologically active components exceed spore concentrations by up to 320-fold [38]. For example, concentrations of airborne trichothecenes carried primarily on fungal fragments smaller than intact conidia were reported to exceed 1300 pg/m^3 in WDBs [11].
3) Young adult mice were used in the rodent studies [33,79]. Younger and older populations, as well as other physically compromised populations, may be more susceptible to exposure-induced inflammation than healthy, young adults.
4) The initial onset of SBS is typically observed in occupants of WDBs following chronic exposure extending to many months [95,97]. Human health risk assessments for SBS should not be based on effect levels from sub-chronic rodent studies without consideration of uncertainty factors. Uncertainty factors include the potential for cumulative effects, toxin accumulation in tissues, and effect threshold shifts to lower levels as protective and repair mechanisms are compromised during chronic exposure. Additional uncertainty factors include interspecies differences in susceptibility and intra-species differences including genetic polymorphisms affecting toxin elimination [97]. Amplification of the pro-inflammatory cytokine response by rising levels of leptin and blockage of the POMC response may also be an important factor in the progression of illness during chronic exposure that may not fully develop during acute and sub-chronic exposures.
5) Previous episodes of SBS from exposure to WDBs may sensitize patients to subsequent exposures. The hypothesis of sensitization is supported by the observation of relapse within 3 days of re-exposure in the current and previous studies [95,97], as opposed to the gradual onset of initial illness reported by the study participants.
6) The potential for additive and synergistic induction of a pro-inflammatory cytokine response by mixture components indicates that human health risk assessments for SBS should be based on studies of exposure to mixtures actually observed in WDBs. Studies should determine the NOAEL for development of an inflammatory response during initial acquisition of SBS following chronic exposure to the mixtures, and the concentration and time dependence of acute exposure-induced reacquisition of SBS following CSM therapy and subsidence of the pro-inflammatory cytokine response.

The ABB′AB design used in the current study yielded data that strongly support the hypothesis that SBS is caused by exposure to WDBs. Although CSM therapy lead to significant improvement in health status, complete recovery was not obtained by some of the study participants. CSM markedly increases the elimination rate of a variety of biotoxins, but does not directly dampen the pro-inflammatory cytokine cascade initiated by exposure. Only research that focuses on the signs, symptoms, and biochemical markers of patients with persistent illness following acute and chronic exposure to WDBs can further the development of the model describing modes of action in the biotoxin-associated pathway and guide the development of innovative and efficacious therapeutic interventions. A more thorough inventory of physiologic and biochemical parameters of affected patients at baseline and during therapy is required, as is a more precise delineation of parametric changes during re-exposure and reacquisition of illness.

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