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Research Article

A Pilot Study on Cultural Stress Anxiety Syndrome, Its Implications on Aging, Gene Expression and Treatment Strategies

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Abstract

Introduction

With advances in technology and our interconnectedness enhanced, the evolution of human life has created a new type of stress, Cultural Stress (CS) and this type of stress has given way to a recognizable syndrome, Cultural Stress Anxiety Syndrome (CSAS), where isolation, anxiety, sedentary lifestyles and depression are the norm and may hasten senescence.

Objective

The purpose of this pilot study is to explore the effectiveness of an inclusive health method to treat CSAS.

Methods

Six female patients were treated for 24 weeks with an inclusive health protocol (internal care, external care and emotional care) designed to reverse CSAS, rejuvenate cellular health and slow related degenerative cell aging processes. Clinical tests such as a comprehensive metabolic panel, blood pressure screenings, BMI/weight, stress test, as well as questionnaires, interviews and journaling were used to establish baseline measures, mid-point and final results. This included cohen perceived stress scale; patient health questionnaire PHQ-9; cultural stress questionnaire; dermatology self-reporting skin questionnaire; appearance questionnaire and video interviews; omnia photos; visia photos; clarity pro evaluations; blood pressure and body composition using an RJL system; skin biopsies and a global gene expression analysis using affymetrix microarrays.

Results

Subjects eliminated the symptoms of CSAS and gene expression examination showed positive results in down-regulation and up-regulation of the genes that influence senescence. Our findings show that inclusive treatment for CSAS can produce positive changes in total wellness and may reduce aging. In specific, we found that treatments that support the cellular water principle theory-which aims to fortify cells and connective tissue, Increase Intracellular Water (ICW) and boost cellular immunity-and address internal, external and emotional stress due to CS, may be useful to encourage maximal youth in aging patients and stave off age-related cellular degeneration.

Discussion

Accordingly, lifestyle and behavioral management therapies that improve cell health and fortification, in concert with medical treatments, may have the most profound effects on CSAS and epigenetic aging factors.

Keywords: Aging; Anxiety; Depression; Epigenetics; Life stress; Psychological stress

Introduction

Epidemiological studies are clear about the deleterious effects of stress on health and aging [1-3]. Stress can cause disease, enhance aging and shorten lifespan [4-6]. Although complex, most kinds of stress are easy to identify: acute stress, episodic acute stress, chronic stress and all types of stress share similarities in how they affect the way the body functions [7,8]. However, more recent examination has produced the need for a new category of stress called Cultural Stress (CS) [9]. CS identifies stress from political, climatological, technological and cultural changes and it is a direct result of our interconnectedness and advances in technology. It is also symptomatic of long commutes, overpopulation, noise pollution, toxin exposure through consumables [10] and the incessant use of smart phones, to name a few things. While CS is manmade, it is largely unavoidable in today's modern society, for those who live on the grid, unless a conscious effort is made to prevent exposure. Even though CS is wholly unnecessary for survival, it can be hard to identify as it is akin to the constant, perhaps unidentifiable, yet equally obtrusive hum of a refrigerator and exists like a ceaseless ringing phone that demands subconscious and conscious management. Patients, most of the time, are conditioned to the continuous stress of CS and are, therefore, unaware that the collection of symptoms and anxiety they experience can be traced back to CS. CS superimposes all other stress types and may even be the cause of acute, episodic acute or chronic stress.

Until now, stress has been hard to define appropriately enough to establish a clear treatment model that addresses all patients' specific needs [9,11]. Currently, about 12% of all Americans take antidepressants to handle stress [12]. Another report indicates that physicians prescribe these drugs for as many on-label uses as they do for off-label use [13]. An NBC report states that one in six Americans currently takes some kind of psychiatric drug [12]. Clearly, stress models of the past fail to identify holistic or inclusive treatments that attack the causes of stress, if they can be addressed. Rather, treatment of stress' symptoms come in the form of vague preventive strategies or prescribed medications, which may also produce unwanted side-effects. Moreover, stress-induced symptoms and exacerbations in patients can present in various ways and to certain degrees, depending on each person's stress threshold, given health, coping skills or even genetics [3]. Indeed, conventional treatments have largely addressed symptoms and are only commenced once health decreases and disorders are present.

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We believe, however, that if CS is decreased or eliminated and resultant CSAS is attenuated, then overall health may improve and senescence could be postponed. Researchers have clearly established a bi-directional relationship between stress and body homeostasis, regardless of the stress type. Systemically, the body is programmed to acclimatize to extrinsic and intrinsic influences and stress causes both adaptive and resistance responses [14]. Because of this, we postulate that treatment of CSAS will not only eliminate the toll its symptoms take on health, but CSAS treatment may also help to alleviate the effects of other types of stress, in specific, chronic stress. Accordingly, we believe that CSAS is a serious, yet unrecognized problem that encourages health declines and may work to hasten age-related processes. While several singular methods have been used in the past to treat stress, CSAS requires comprehensive, multidisciplinary treatment. As such, within this pilot study, we hypothesized if one can reduce stress including CS and resultant CSAS to improve overall health by engaging in inclusive health activities (activities that function on the cellular and mechanistic level, influence intracellular water balance and genetic expression), then one may benefit from positive health outcomes that influence senescence.

Methods

Our objective was to stave off stress caused by CS. Our pilot study began with a simple understanding of the particular root causes of CS. We have found that simply identifying the causes of CS helps our patients, therefore, we educated our study's participants so they could better understand limits and disconnect from technology and other extrinsic stressors that deliver CS. We also devised a comprehensive program including internal, external and emotional treatments to assist patients with CSAS and work to reduce its effects on the cell processes, which, in effect, could reduce the signs of aging and slow the progression of aging [15]. Our treatment method began with an understanding of cell health, immunity, intracellular water and an awareness of how the symptoms of CSAS affect cellular functions and encourage senescence. Hence, we defined CSAS for our study's participants and evaluated their collected symptoms to create individualized programs that addressed each participant's specific needs.

Defining cultural stress anxiety syndrome

An inventory of current affairs will produce an ample supply of CS examples. We asked participants to consider the political situation and acts of terror and civil unrest and discussed the new type of stress: CS. We suggested a simple cruise through regular social media sites, which offered many examples of negative sentiments and hostility from every avenue and every viewpoint. Our patients began to find their current exposure to CS through technology was not only daily, but up-to-the-minute. Because of this constant exposure, we believe that it is possible that more Americans than ever are experiencing CS and its resultant CSAS.

Understanding the effects of stress including CSAS

Generally, the body reacts to stress with increased neural, cardiovascular and metabolic activity, coupled with higher sodium retention, added nervous sweating, dry mouth and GI track disorders [2]. Stress also enhances inflammatory cascades, endocrine imbalances and depletes the integument system's antimicrobial barrier that protects against cutaneous infection, reactivates skin disorders like eczema, stimulates acne and intensifies allergy symptoms [16,17]. These symptoms as well as the many other symptoms associated with stress are the same in CSAS. CSAS, however, is associated with depression caused by profound isolation, constant and unabated CS exposure, anxiety and sedentary lifestyles [9,18,19]. In CSAS, symptoms of isolation, anxiety, sedentary lifestyle and depression overlap, work together and enhance each other. The symptoms may present singly, in combination, at different intervals or levels and one or more symptoms may mask the others. These symptoms and constant CS influencers may also be the primary sources of stress and/or function to magnify all other types of life stress outside of CSAS, both intrinsic and extrinsic [6,20]. Knowing this, treatment of CSAS is all-encompassing, for each symptom (isolation, anxiety, sedentary lifestyle, depression) even if a symptom is not present. In this case, treatment is preventive and simplifies therapies as a quashed symptom prevents the rise or increase of another in CSAS. Going further, the symptoms of CSAS may precipitate many deleterious health effects including sleeplessness, obesity, asthma, poor wound healing, etc., [21-24]. The greatest marker of CSAS, however, is isolation.

Isolation

Today's technology allows people to receive constant reminders of the news, social media, scheduling, etc. Texting also permits constant contact with others, which is a condensed and perhaps deteriorated form of communication. In addition, long commutes require isolation from family and friends and disrupt traditional family time, meals and extracurricular activities as modern commuting consumes extra time available for these events. Ironically, this constant awareness of daily life, commuting and communication has directly enhanced our isolation from face-to-face time with others and real, expanded communication that form the basis for relationship building. Early studies theorized whether the use of personal computers could actually reduce isolation for the elderly and while short-term results showed positive outcomes, the long-term results were inconclusive [25]. More recent study shows that the result of constant technological connection is an overwhelming remoteness or loneliness, which, in advancing age, can work against patients who need network support to cope with life events [26]. This connectivity has also been found to result in sleeplessness, increased perceived stress and may even be the catalyst for depression [27]. The lack of real friendships, companionship and the support that comes from these relationships has been correlated to poor health prognosis, quality of life, an escalation of age-related deteriorations and even death [28,29]. In advanced age, when there is already a chronic illness, isolation can encourage worsening health [30]. Isolation is also linked to cardiovascular disorders and maladaptive behaviors such as smoking, alcohol and substance abuse, as well as overeating [31]. Notwithstanding, isolation is generally linked with anxiety.

Anxiety

Anxiety is also another symptom of CSAS. Within the context of CSAS, anxiety exists concurrently with and may be enhanced by isolation. While anxiety is a normal reaction to stress, constant anxiety or anxiety disorders can interfere with normal life activities. There are several types of anxiety disorders such as generalized anxiety disorder, social anxiety and panic disorder. While the direct cause of these anxiety disorders is unknown, they are mental illnesses that may exist as a result of a combination of both intrinsic and extrinsic factors. As such, it is plausible that unrelenting CS and isolation may produce and even lead to anxiety disorders in those who are most at risk of developing them. Along these lines, recent research shows that Post Traumatic Stress Disorder (PTSD) is a growing epidemic, as it is not just experienced by those who have experienced live combat while serving in the

military. PTSD can be the result of community calamities, terrorism and can also be the result of constant stress from the endless news cycle covering such events [32]. Often, PTSD, which can be experienced at any age, accompanies blood pressure irregularities, depression and sleeplessness [33]. Notably, a treatment for anxiety disorders including PTSD is talk therapy or support groups, as this reduces isolation and this further outlines the overlap that exists among CSAS's symptoms.

Sedentary lifestyle

Modern society demands the constant use of technology. As a result, people are more sedentary than ever in the office, at home and at school. One study's analysis shows that "1 in 4 white U.S. adults spends about 70% of their waking hours sitting, 30% in light activities and little or no time in exercise" [34]. Prolonged sitting has likely contributed to the increased rates of obesity and diabetes in our nation. According to the World Health Organization (WHO), all causes of mortality are doubled with a sedentary lifestyle. This includes, "double the risk of cardiovascular diseases, diabetes, obesity and increased risks of colon cancer, high blood pressure, osteoporosis, lipid disorders, depression and anxiety" [35]. According to the WHO's report, globally, approximately 60% to 85% of people maintain sedentary lifestyles. This fact, says the organization, makes it a top, worldwide public health concern that it is "insufficiently addressed," further, the organization estimates that "nearly two-thirds of children are also insufficiently active" [35]. Notwithstanding, patients who are largely sedentary usually suffer from a degree of isolation and resultant depression.

Depression

Within CSAS, depression and isolation go hand-in-hand. Social withdrawal is a pitfall to depression as is lack of exercise, rumination, alcohol and substance abuse, overeating for sugar surges and negative thinking [36]. Depression is also strongly linked to anxiety. It is a known fact that those who are aging are at the highest risk of developing depression; however, depression is not a natural part of aging. Because life-changing or constant stress is a catalyst to depression, it is conceivable that constant exposure to CS can cause depression. CS stimulates a constant stress response in the body and this may precede down spiraling episodes of anxiety and depression and initiate CSAS.

Attack root causes of cultural stress

Our system of treatment promoted inclusive health therapies that attacked the root cause of CS for our study's participants, whether it was computer use, TV watching, commuting, noise pollution, toxicity, etc., and replaced these items with positive insights and informal seminars or talks, where they were able to meet every other week and hear a lecture and discuss events and stressors. In addition, the participants were provided literature that explained cultural stress as well as how to increase cellular water through whole health strategies involving lifestyle changes. The subjects were given time to reflect on themselves and to release stressful burdens through journaling. In effect, we addressed the symptoms of CSAS from many avenues (internal, external and emotional) through methods that enhance cellular health, using the cellular water principle theory and behavioral practices that promote mental well-being. While the method we employed only included a few therapies, new therapies and treatments will likely be added with future examinations.

The cellular water principle theory

At the foundation of our CSAS treatment, we advised patients to create the best cellular health possible. This included treatment at the cellular level to address weakened or damaged cell membrane walls and restoration of intracellular water, among other things. Cell repair, fortification and health forms the foundation of the cellular water principle theory (Figure 1), which builds on Nagy's membrane hypothesis on aging. Nagy postulated that cell aging is the result of wear-and-tear, cell damage, intracellular waste accumulation and cell membrane permeability as the result of ROS-induced cross-linking and the residual heat formed during heat discharge of the resting potential [37-39]. Simply, ICW loss precipitates cell and connective tissue deterioration and damage, regardless of the cause [6,20]. When ICW is replaced and cells restored and fortified, damage is reduced and even corrected in organs and tissues [40]. This only occurs when the body receives an abundance of nutrients so cells can be flushed with the components they need to commence repair functions that promote immunity and, as a result, improve health [6,20,41]. Therefore, once evaluations for baseline measures were complete, treatment for CSAS started with a nutrient rich diet and supplementation as well as a comprehensive, inclusive health protocol including internal, external and emotional treatments.

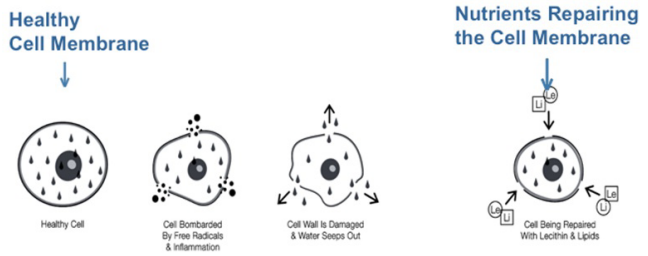

Figure 1: Cell deterioration and fortification. This figure illustrates the cellular water principle theory.

Evaluation and Treatment

Beyond therapies that improve ICW through the cellular water principle theory, patients need to be evaluated internally, externally and emotionally to determine a course of treatment that addresses specific needs. Our pilot study was aimed at adult patients (under 55 years of age). In our study, six female patients between the ages of 46 and 53, underwent a 24-week program to reduce CSAS and improve health, wellness and explore treatments that may offer implications with regard to genetic expression and aging, however, it is important to clarify that the selected patients did not represent the elderly group. Through evaluations that included measuring all attributes including head and belly circumference and tests, a roadmap for individualized treatment of the study's participants with CSAS began to emerge. In general, the 24-week study included the following three pronged inclusive health approach, which combines multidisciplinary interventions including external care, internal care and emotional care:

External care: To address innate immunity; strengthen cutaneous defenses to external influences or invaders. At the start of the study and each day after, the subjects performed a daily skin care regimen. External care included patient-specific cleansers, treatments and hydration to repair damage and protect and fortify the skin's barrier function and connective tissue from external factors such as UV rays as well as collagen-deposition and -formation encouraging agents and treatments.

Internal care: To encourage adaptive immunity. Diagnostic tests at day one, assisted in determining systemic disease such as endocrine system or vascular disorders, so therapeutic, systemic care could be initiated. Therapeutic care during the first week of treatment included

nutritional guidance on how to eat a low-acid diet replete with vegetables and fruits, lean meats and minimally processed foods. It also included formal physical activity classes two times a week during the entire study such as hiking and dance classes and encouraged participants to increase activity at home to reduce sedentary lifestyles. Also on day one of the study, our subjects commenced needs-based prescription medication and supplements including sleep-encouraging supplements:

- A supplement designed to encourage restful sleep including chamomile, valerian, lavender, melatonin and GABA
- Multivitamin and mineral supplement. An iron-free, comprehensive and balanced formula containing all the major vitamins, minerals and trace minerals
- Antioxidant supplement formula
- High-potency B complex supplement providing all eight essential B vitamins: thiamine (B1), riboflavin (B2), niacin (B3), pantothenic acid (B5), pyridoxine (B6), folic acid (B9), cyanocobalamin (B12), biotin (B7)
- Essential fatty acid supplement providing omega-3 fatty acids. This was in the form of fish oil, flaxseed oil, or ground flaxseeds added to food, or in capsule form
- Lecithin supplement. Soy lecithin granules were sprinkled on or added to foods or added via liquid soy lecithin in capsule form
- Glucosamine
- Calcium supplement for bone health between 1000 mg to 1500 mg of calcium with vitamin D daily, depending on the subject's dietary intake of calcium

Emotional care: To support psychological and social balance. Stress reduction "feel-good" services such as those provided within the spa environment were used, as well as activities like yoga, support group participation, art classes, positive living lectures, daily reading of life-affirmation flashcards and counseling to nurture the psyche. Eleven insight cards, a journal and an explanation of CS were given at the beginning of the study and subjects were asked to download an app that had more life-affirming quotes and artwork. The insight cards were used as a tool to promote well-being and reduce CS. Focused attention to the cards was recommended twice a day and journaling was once a day during the entire study.

During the 24-week study, participant performed a self-assessment of emotional and physical well-being by completing several brief questionnaires. In-office tests were also performed:

- Cohen perceived stress scale
- Patient health questionnaire PHQ-9, cultural stress questionnaire by Murad
- Dermatology self-reporting skin questionnaire
- Appearance questionnaire and video interviews
- Omnia photos, visia photos, and clarity pros evaluations
- Blood pressure and body composition with an RJL system
- Blood tests (complete metabolic panel)
- Skin biopsy
- Global gene expression analysis using affymetrix microarrays

Blood and skin biopsy samples were collected at baseline, 12 weeks and 24 weeks to measure every single gene in the body. Our pilot study focused on the 12-week dataset as this analysis contains the use of the benjamini-hochberg FDR correction. Accordingly, there is a large amount of data with the FDR correction in both the 12-week blood and skin sample datasets.

A body composition using the RJL system allowed scientific data for cellular health among the subjects. The data included body fat, fat-free mass, intracellular water, extracellular water and total-body water percentages. Clarity pro, an analytical machine, monitored the progression of the subjects' skin condition throughout the study. The technology allowed baseline, week 12 and week 24 data comparisons in order to detect any changes topically for each individual subject. For further investigation regarding gene expression, the samples from the blood tests and skin biopsies were analyzed. As expected, the blood and skin datasets had little in common. Based on the small overlap in specific genes, each tissue was further analyzed independently (Figure 2).

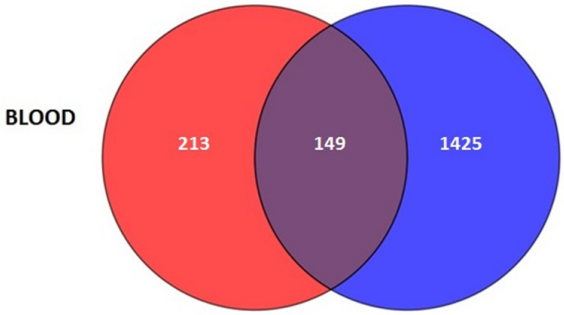

Figure 2: Comparison of genes in the blood and skin datasets. This figure shows the small overlap in specific genes.

Global gene expression analysis was carried out using affymetrix microarrays to identify gene expression changes correlated with improved health outcomes as a result of the program. Affymetrix microarrays contain approximately 45,000 transcripts representing the ~23,000 human genes and are an ideal method for screening the entire genome. Samples were processed according to standard affymetrix microarray protocols.

Data analysis was conducted using gene spring software. Paired t-tests were performed to identify statistically significant changes in gene expression between the following time points:

- 12 Week versus Baseline (skin)
- 12 Week versus Baseline (blood)
- 24 Week versus Baseline (skin)
- 24 Week versus Baseline (blood)

Patients were given ongoing questionnaires and interviewed on the status of their current stress levels. This was done both orally and through written responses, video interviews and journaling twice daily. Finally, an assessment of current exposure to CS was necessary to determine external influences that may enhance CSAS. Depending on stress levels and symptoms determined, medical interventions were explored with the objective of only short-term use and discontinued once symptoms reduced enough and general health and cellular immunity improved. Also, patients were instructed in how to avoid CS so as not to encourage CSAS, with the following tips [20]:

Tips for reducing cultural stress

- The first step in reducing CS is to determine its sources. Once the sources are identified, develop a plan of action to reduce their impact
- Practice being mindful: Take some time each day to meditate or be quiet and enjoy the simple rhythms of life
- If you are stuck in traffic and late for an appointment, accept the fact that you can't control the situation. One thing you can control is how you react to these situations. Try to make the best of it. Why have a bad day when you can have a good day?
- Exercise regularly: Go for a walk, do yoga or take an exercise class. Being physically active, even for just a few minutes can make a difference in the way you feel
- Nourish your body for optimum health: Make it a habit to avoid the Standard American Diet. Get foods that encourage and increase the water content in your body - a diet full of whole grains, fresh fruit and vegetables, good fats and proteins. Take a nutritional supplement to fill the nutritional gaps in your diet
- Get a good night's sleep: Americans sleep less than people in any industrialized country in the world. You need seven to nine hours of sleep every night to fully restore the body. Don't lose sleep; find the time to recharge your body at night so you have the energy to face the challenges that come up every day

Mind-Body connection

In our study, patients were encouraged to use the mind-body connection or Neuro-Immuno-Cutaneous-Endocrine network (N.I.C.E.), to reduce stress and increase health, bi-directionally [42,43]. This included the use of mind-relaxing therapies to improve skin conditions and general health and draw on skin conditioning therapies to improve mind relaxation. The study's participants used massage and facials every other week. It also included mental awareness techniques to rejuvenate the mind. Simply, patients were encouraged to try to return to a younger mentality like that of a toddler and reject, within reason, the social demands and worries of modern society. Through conscious awareness and the prevention of mental rumination, patients reported they felt they were living more at ease. While this technique was harder for some than others, many times, the simple suggestion or permission from a physician to let go and disconnect started the processes of relaxation and reduction of stress.

Aside from the mind, the body may be addressed with exercise techniques that reduce stress and improve circulation, as well as tackle sedentary lifestyles. Our study's participants were encouraged to join support groups, cooking classes, walking clubs, exercise groups two to three times per week and sign up for new experiences such as dancing, art, or photography classes. They were challenged to do more things like hiking and attend cooking classes. All six participants met twice a week with a trainer to exercise. They also attended an art therapy class that encouraged coloring outside the lines and laughter. Pet adoption was also suggested as pets have been shown to help reduce stress.

Results

CSAS initiates the body's immune response, oxidation and a decrease in telomerase activity. Specifically, CSAS impairs the immune response and this initiates declines in the body and its organs as oxidation is permitted to accumulate, reducing telomerase activity and shortening telomere length. These reductions in telomerase activity and length are markers of cell aging and have implications regarding lifespan [4-6,20]. These are not the only declines that occur, however, as CSAS reduces cellular water homeostasis. CSAS causes water to be lost through perspiration and at the same time, CSAS causes inflammation in the body [44]. Where there is a lack of inflammatory mediators, connective tissue and cells may become damaged or weakened so vital, nutrient-rich cellular water escapes into the extracellular matrix. This damage encourages the likelihood of disorders, disease and even aging [45-48]. In patients who are advanced in age, intracellular water is reduced and even more dramatically if the patient suffers from illness or disease [49]. Understanding these processes, it can be extrapolated that decreased intracellular water allows CSAS to cause more damage at the cellular level and can accelerate aging processes and disease. The most prominent gene expression results were seen during the midpoint of the study.

At the midpoint of the study, based on self-assessments, the participants also reduced overall depression from 33% to 24%. Intracellular water percentages increased within the overall group. Clarity pro analyses also indicated noticeable improvements in facial skin. The women were able to reduce wrinkles, sun damage, acne, and UV damage. With such results, an increase in confidence allowed the women to handle new challenges in their daily lives, such as promotions and even address issues preventing them from being better mothers. All in all, the women successfully completed the program with various internal, external and emotional improvements. The midpoint results, which had the most prominent change among the women, showed NFAT5 activity was reduced. In fact, biopsies showed 1,425 genes were expressed in a positive way to slow age-related deteriorations. In the blood samples, 200 gene-expression markers showed reductions and increases in a positive way, as well. Of those 200 markers, 100 overlapped with tissue sample results and both tissue and blood samples produced identical positive results (Table 1).

Number of Two-Fold Genes					
		12 Wk-vs-Wk 0		24 Wk-vs-Wk 0	
		No correction	With FDR	No correction	With FDR
Blood	Increased	240	3	60	0
	Decreased	3357	359	168	0
	Total	3597	362	228	0
Skin	Increased	156	61	80	0
	Decreased	2532	1513	168	0
	Total	2688	1574	248	0

Table 1: Number of statistically significant genes with a fold-change value greater than 2.0.

Paired t-tests with a p-value cut-off 0.05 were performed using gene spring software. Most reviewers of peer-reviewed journal require statistics performed with a False Discovery Rate (FDR) correction. However, studies with small sample sizes often do not support the use of this correction. The tables above show data that was generated with and without the use of the Benjamini-Hochberg FDR calculation. A fold change value of 2.0 was used to filter the datasets, as this is a value that is considered to be biologically relevant by most researchers.

Summary of key gene expression findings

- NFAT5 (Nuclear Factor of Activated T-cells 5) was decreased in both the blood and skin after 12 weeks of participation in the inclusive health
- NFAT5 is a molecule that is increased in response to hypertonic stress
- NFAT5 is a transcription factor that regulates multiple biological functions and genes

- A decrease in NFAT5 at 12 weeks suggests that the levels were higher at baseline. This is consistent with the idea that at baseline the cells were exposed hypertonic stress that was diminished by participation in the inclusive health protocol
- Each of the datasets (blood and skin) contains multiple genes that regulate or are regulated by NFAT5

Reduction of NFAT5 during the period of the study with the most changes within the three-facet approach indicates a correlation between inclusive health therapies and whole body wellness (Figures 3-6). Changes in environmental conditions have been scientifically proven to change protein expression due to increases in euchromatin and heterochromin in the nucleosome structures of the DNA. While such changes in epigenetics can have positive or negative effects on individuals health-wise such as the inactivation or activation of tumor suppression genes like connexin [50], the CSAS inclusive health program seemed to have only positive effects on reducing negative transcription factors such as NFAT5. These results showed that an inclusive protocol based on cellular health could reverse CSAS, improve health and delay cellular deteriorations including those due to aging.

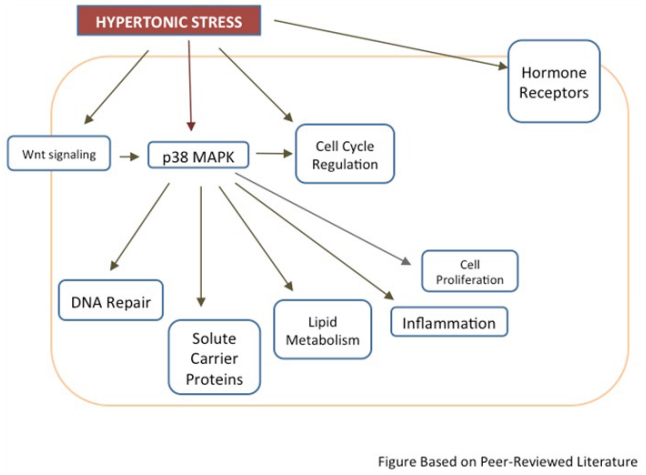

Figure 3: Normal responses to hypertonic stress. This figure illustrates the normal reaction to hypertonic stress on biological function.

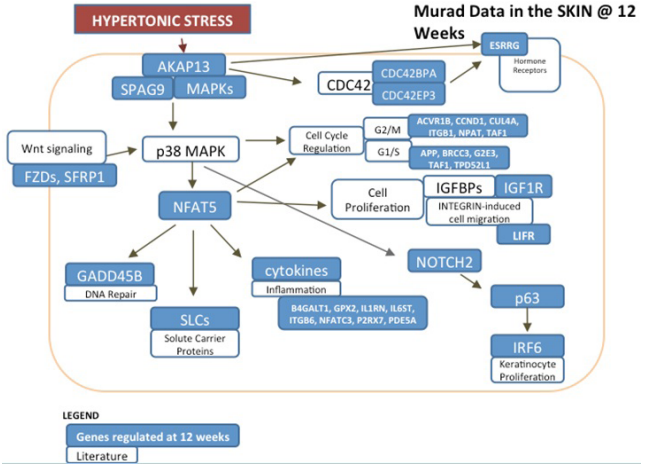

Figure 4: Responses to hypertonic stress in skin biopsies. This figure illustrates the responses, within skin samples, to hypertonic stress after 12 weeks of treatment.

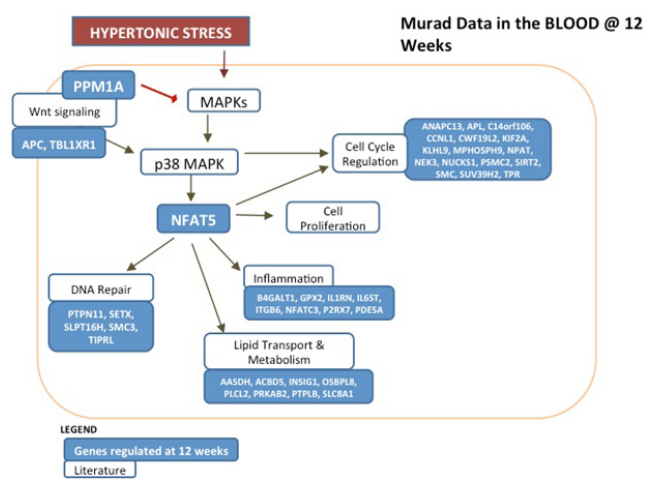

Figure 5: Responses to hypertonic stress in blood tests. This figure illustrates the responses, within blood samples, to hypertonic stress after 12 weeks of treatment.

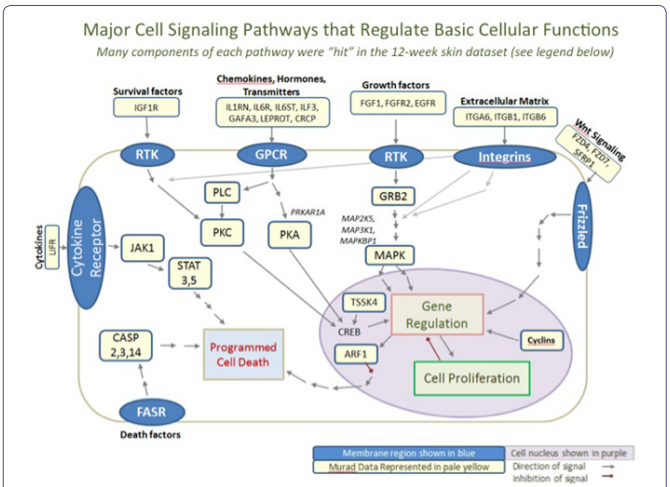

Figure 6: Regulation of biological pathways. This figure summarizes the data collected and shows that multiple, major mechanisms were contained within the dataset(s).

Hormone regulation

The following genes were decreased in the skin:

AR [Androgen Receptor]

CRCP [CGRP Receptor Component]

ESRRG [Estrogen-Related Receptor Gamma]

GLCCI1 [Glucocorticoid Induced Transcript 1]

GNRHR2 [Gonadotropin-Releasing Hormone (Type 2) Receptor 2]

GRB2 [Growth Factor Receptor-Bound protein 2]

IGF1R [Insulin-like Growth Factor 1 Receptor]

LEPROT [Leptin Receptor Overlapping Transcript]

PDGFA [Platelet Derived Growth Factor Alpha polypeptide]

PGRMC1 [Progesterone Receptor Membrane Component 1]

PRLR [Prolactin Receptor]

PTGFR [Prostaglandin F Receptor]

THRAP3 [Thyroid Hormone Receptor Associated Protein 3]

Additional mechanisms identified using biological pathways analysis

For this study, we used pathways available in gene spring and the NIH (DAVID) website to identify additional biological pathways that were influenced by the inclusive health protocol. Figure 5 shows a summary of the data collected and table 2 lists the genes in the major cell signaling pathways.

Gene Id	Gene Name
IGF1R	Insulin-like Growth Factor 1 Receptor
IL1RN	Interleukin 1 Receptor Antagonist
IL6R, IL6ST	Interleukin 6 Receptor & Interleukin 6 Signal Transducer
ILF3	Interleukin Enhance Binding Factor 3
GAFA3	FGF-2 Activity-Associated Protein 3
LEPROT	Leptin Receptor Overlapping Transcript
CRCP	CGRP Receptor Component
FGF1	Fibroblast Growth Factor 1
FGFR2	Fibroblast Growth Factor Receptor 2
EGFR	Epidermal Growth Factor Receptor
ITGA^	Integrin, Alpha 6
ITGB1	Integrin, Beta 1
ITGB6	Integrin, Beta 6
FZD4, FZD7	Frizzled Homolog 4 & 7
SFRP1	Secreted Frizzled-Related Protein 1
PLCB4, PLCZ1	Phospholipase C, Beta 4 & Zeta 1
CREB	(TSSK4) Positive Regulation of CREB Transcription Factor Activity
GRB2	Growth Factor Receptor-Bound Protein 2
CASP2, CASP3, CSAP14 and CFLAR	Caspase 2,3 & 14, and the CASP8 and FADD-like Apoptosis Regulator
JAK	Janus Kinase 1
STAT3	Signal Transducer and Activator of Transcription 3
STAT5	Signal Transducer and Activator of Transcription 5B
ARF1	ADP-Ribosylation Factor 1
MAP2K5, MAP3K1, MAPKBP1	Mitogen-Activated Protein Kinase Kinase 5, Mitogen-Activated Protein Kinase Kinase 1, Mitogen-Activated Protein Kinase Binding Protein 1
LIFR	Leukemia Inhibitory Factor Receptor
CCND1, CCNG2, CCNK, CCNL1	Cyclins D1, G2, k & L1
PRKAR1A(PKA)	Protein Kinase, cAMP-Dependent, Regulatory, Type 1, Alpha
PRKCI(PKC)	Protein Kinase C, Iota

Table 2: Genes listed in the major cell signaling pathways.

Blood sample pathways

Due to the smaller size of the blood cell dataset, only a few biological pathways represented in the software programs were "hit." The following pathways were impacted in the blood cell dataset: programmed cell death; inflammation and immunity; and major transcription factors.

Major transcription factors regulated in blood

Additionally, several major transcription factor molecules were regulated in the blood samples. Transcription factors are molecules that bind to DNA and turn on or turn off gene expression. Some of the major transcription factors included in the blood samples dataset: SP4; CREB1; JAK3; GTF2A1.

Skin sample pathways

The skin cell dataset was much larger-many more biological pathways represented in the software programs were "hit" with this dataset:

- Cell-cell adhesion
- Extracellular matrix regulation
- Epidermal growth factor pathway
- Integrin-regulated matrix functions
- Cell proliferation and differentiation
- TGFβ-induced regulation of the cell cycle
- Wnt regulation of the cell cycle
- Cellular metabolism
- Hormone receptors

Cellular metabolism

In addition, multiple genes associated with cellular metabolism were contained with the skin dataset. The following genes were decreased in the skin:

- Several ATPase family genes: ATP1B1, ATP13A3, ATAD2B, ATP2B4, ATP6V1A, ATP6V1C1, ATP6V1G2
- Cellular respiration genes: CYCS [Cytochrome C], UQCRC2 [Ubiquinol-Cytochrome C Reductase Core protein II], NDUFS1 [NADH-Coenzyme Q Reductase]
- ~20 solute carrier /transporter molecules: SLC2A4 [Solute Carrier family 2 (facilitated glucose transporter), member 4], SLC17A5 [Solute Carrier family 17 (anion/sugar transporter), member 5], Other membrane transporters: SLC10A1, SLC11A2, SLC13A1, SLC16A10, SLC16A7, SLC23A2, SLC26A2, SLC26A2, SLC35E1, SLC38A1, SLC38A4, SLC38A7, SLC39A6, SLC39A8, SLC39A8, SLC46A1, SLC4A1, SLC4A4, SLC7A8, SLC8A1
- ~40 zinc-finger related genes (DNA transcription): ZBED6, ZBTB33, ZBTB38, ZBTB40, ZBTB43, ZC3H11A, ZDHHC21, ZEB1, ZEB2, ZFP106, ZFR, ZFYVE9, ZKSCAN1, ZMYM5, ZMYM6, ZMYND11, ZNF148, NF22, ZNF248, ZNF254, ZNF264, ZNF275, ZNF292, ZNF395, ZNF396, ZNF397OS, ZNF434, ZNF440, ZNF479, ZNF480, ZNF496, ZNF551, ZNF567, ZNF621, ZNF641, ZNF652, ZNF704, ZNF91, ZSCAN18
- LIPE [Hormone-sensitive lipase]
- STAT3 and STAT5B [Signal Transducer and Activator of Transcription 3 and 5B]

Skin function: Barrier and extracellular matrix integrity

Many genes that regulate integral skin functions were included in the skin dataset. The following genes were decreased in the skin:

COL4A3 [Collagen, Type IV, Alpha 3]

COL6A1 [Collagen, Type VI, Alpha 1]

DEFA1 [Defensin Alpha 1]

BMPR2 [Bone Morphogenetic Protein Receptor, Type II (Serine/Threonine kinase)]

CDH1 and CDH4 [Cadherin 1 and 4]

DCT [Dopachrome Tautomerase]

DEFA1 [Defensin Alpha 1]

DSG2 [Desmoglein 2]

ITGA6, ITGB1, ITGB6 [Integrins A6, B1, and B6]

LAMA4 [Laminin A4]

PLOD2 [Procollagen-Lysine 2-Oxoglutarate 5-Dioxygenase 2]

S100A1, S100B [S100 Calcium Binding Proteins A1 and B]

SERPINB13 [Hurpin, Serine Protease Inhibitor 13]

TIMP3 [TIMP Metallopeptidase Inhibitor 3]

TNXB [Tenascin XB]

Discussion

This pilot study offers correlations on decreased intracellular water, cell deterioration, healthy activities and stress and its results accurately indicate the importance of the epigenetic factors of aging, such as the environmental implications in the lifestyles and the management of behavioral therapies, but only in a small sample of six adult women. A full review of the scientific literature at an international level and the use of a sample representative of gender, age groups, educational levels, racial groups, cultural groups and diverse environmental context across the sexes and age groups, as well as a consultation of web of science databases and scopus would help to better define inclusive health protocols for CSAS. Admittedly, this early study does not represent an exhaustive review of the scientific literature at the international level, nor a complete review of the complementary studies on environmental stress anxiety in aging and its implications with unhealthy environments, including isolation and sedentary lifestyles and from the perspectives of environmental gerontology and epigenetics. The results found, however, offer new thoughts on how CS and its related CSAS might be treated with an inclusive health protocol, which further, international studies may revisit and build on.

As technology increases and the world becomes more connected, it is probable that stress sources will only increase and symptoms in patients will multiply. CS is pervasive, unrelenting and intrusive. Patients must consciously recognize it, turn it off or disconnect and divert energies to mindful relaxation, as CS is unnecessary for survival. Through technological advances, it seems very possible that future therapy for isolation and stress may include the addition of artificial intelligence in the form of robots for companionship. The pharmaceutical industry may also provide a better solution for depression that includes a rapid-acting medical drug intervention. This intervention could be useful to halt the effects of life-changing, severe emotional stress and, in emergency situations, stop thoughts of suicide within minutes. As an aside, it is clear that extrinsic stressors have caused life-altering mental disorders when considering the 24% increase in suicide between 1999 and 2014 and the continually growing rates [51]. We believe that unabated, CSAS not only ages patients, it may put them at increased risk for suicide. Using a comprehensive system that features therapies in line with the cellular water principle theory may help to undo the intrinsic effects of stress, while mental therapies can help patients deal with extrinsic CS and emotional instability. While this study offers compelling pilot data, caution should be taken as it only included six subjects. Larger, more long-term studies are warranted to help physicians treat aging patients struggling with living in today's stressful world.

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