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RESEARCH ARTICLE

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Does stress response axis activation differ between patients with autoimmune disease and healthy people?

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Abstract

Many studies have shown that patients with autoimmune disease present a hypoactive hypothalamic-pituitary-adrenal (HPA) axis, but the results are controversial. Our objective was to study differences in stress response axis activity between patients with autoimmune disease and healthy people. The study sample consisted of 97 women divided into four groups: 37 healthy women (HW), 21 with systemic lupus erythematosus (SLE), 21 with Sjögren's syndrome (SS), and 18 with systemic sclerosis (SSc). After being exposed to a stress task, participants' skin conductance and salivary cortisol levels were measured in order to assess their response to psychological stress. Diurnal cortisol concentrations were assessed by measuring salivary cortisol in samples collected five times over one day. In addition, self-administered questionnaires were used to assess psychological variables. A time × group interaction effect was found ($p = 0.003$) in salivary cortisol secretion in response to stressful challenge. The healthy group presented normal activation, the SS and SLE groups showed no activation, and the SSc group presented a similar activation pattern to the HW group, except at the time of recovery. Total cortisol production (AUCg) was higher in the SSc group than in the HW group ($p = 0.001$). Differences were also observed in the cortisol AUCg collected over one day between healthy women and patients with SLE ($p = 0.004$) as well as with SSc ($p = 0.001$): women with SLE and SSc presented higher total hormone production than healthy women. Patients with autoimmune disease present a different HPA axis response, which may contribute to the harmful effects of stress in these diseases.

KEYWORDS
cortisol, HPA axis, Sjögren's syndrome, stress, systemic lupus erythematosus, systemic sclerosis

1 | INTRODUCTION

There has been a recent surge of interest in the relationship between psychological stress and autoimmune diseases. Moreover, several studies have demonstrated a relationship between stress and both hormonal and etiological factors in the pathogenesis of autoimmune diseases (McCray & Agarwal, 2011). One characteristic that is common to all these diseases is the exacerbating effect of stress, since stress worsens symptoms and even precipitates disease (Stojanovich, 2010). This effect has been widely investigated in systemic autoimmune diseases such as systemic lupus erythematosus (SLE), but much less so in conditions such as Sjögren's syndrome (SS) and systemic sclerosis (SSc).

Montero-López et al. (2017), compared salivary as well as hair cortisol levels between patients with SLE, patients with SS, patients with SSc, and healthy persons. The results showed that patients with autoimmune diseases presented greater short and long-term HPA axis activity than healthy women.

The main results of studies on the effect of stress in SLE indicate that daily stress is associated with worsening symptoms, but not stressful life events (Pawlak et al., 2003; Peralta-Ramírez et al., 2004). Furthermore, fluctuations in the amount of stress and interaction with the −1019G allele in the 5-HTR1A gene are more closely related to this deterioration than high levels of stress maintained over time. It has thus been shown that in situations of stress, the change that occurs in the immune response pattern of patients with SLE differs from that of healthy people (Roussou et al., 2013).

With respect to people with SS, studies have shown that prior to disease onset, patients present high stress levels, which may have been caused by negative life events. Adverse childhood experiences and/or an absence of adaptive coping strategies in people with SS are related to a higher rate of psychological disorders compared to SLE patients and healthy people. Moreover, they often present difficulties in adapting to stressful life events, which is a risk factor for mental disorders (Shelomkova et al., 2013).

In the same line, studies on the effects of psychological stress on SSc agree that this stress is a risk factor for SSc disease onset and exacerbation (Matsuura et al., 2011; Newton et al., 2012).

Nevertheless, the mechanisms underlying the deterioration caused by psychological stress are not entirely clear (Freier et al., 2010). Several studies have reached different conclusions regarding the degree of activation of the sympathetic nervous system (SNS) and the hypothalamic-pituitary-adrenal (HPA) axis, that is, the main axes involved in stress response.

With respect to the SNS, its activation in response to psychological stress in people with autoimmune diseases has been found to be similar to that of healthy people (Finan & Zautra, 2013) and may even present higher levels of activation of the sympathetic tone at rest (Straub et al., 2005).

With respect to the HPA axis in autoimmune diseases, Munck et al. (1984) have suggested that a proper secretion of glucocorticoids increases innate immunity as an adaptive response to stress. However, if this increased immunity is maintained, it can lead to autoimmunity, and therefore the HPA axis suppresses the immune system, and hence the risk of autoimmunity through the secretion of cortisol. Several studies have found that psychological stress in autoimmune diseases causes HPA axis activity alterations, producing resistance to glucocorticoids and an imbalance in cytokines, which would explain the onset of autoimmune diseases due to faulty cortisol secretion (Delevaux et al., 2013). Yet, this phenomenon is not generalised since no such imbalances have been observed for other autoimmune diseases such as rheumatoid arthritis (RA) (Finan & Zautra, 2013). These findings were confirmed in a study by Straub (2014), who found that HPA axis activity in patients with RA was similar to that of healthy people when exposed to a laboratory stressor. However, HPA axis activity is expected to be greater in patients with a chronic disease and inflammation. To compensate for this lack of HPA axis activation necessary in cases of inflammation, the SNS was activated chronically, which led to a lack of synchronisation between the two axes as a measure of immune system neuroendocrine control (Straub et al., 2010).

In short, no consensus has been reached regarding the behaviour of the axes involved in the response to psychological stress in autoimmune patients. The desynchronisation of these axes may contribute to exacerbating symptoms in these diseases (Freier et al., 2010), implying further harm to patients. Therefore, the study objective was to identify any differences between healthy women and women with systemic autoimmune disease (SLE, SS and SSc) regarding the activity of the axes involved in the stress response. To this end, participants were exposed to a situation of acute laboratory stress: they had to speak in public via virtual reality while the axes involved in stress response were assessed. Skin conductance was measured to assess SNS activation and salivary cortisol to evaluate HPA axis activity. In addition, we assessed diurnal cortisol concentrations based on salivary cortisol levels measured at five different times over the course of one day.

2 | METHODS

2.1 | Participants

Participants were drawn from a larger study on stress and autoimmunity (Montero-López et al., 2017). Ninety-seven women participated in this study. Thirty-seven were healthy controls (HW), twenty-one had Sjögren's syndrome (SS), twenty-one had systemic lupus erythematosus (SLE) and eighteen had systemic sclerosis (SSc). The women in the three autoimmune groups were recruited by medical from the Systemic Autoimmune Disease Units (Internal Medicine Services) at the Virgen de las Nieves University Hospital and the San Cecilio Clinical Hospital in Granada, Spain. All patients included met the revised SLE diagnostic criteria of the American College of Rheumatology or Systemic Lupus International Collaborating Clinics criteria (Hochberg, 1997). For the SS diagnostic, patients were classified according to the 2012 American College of Rheumatology classification criteria (Shiboski et al., 2012). Finally, an SSc diagnosis was considered when the patients met the ACR/EULAR criteria (Van

de Hoogen et al., 2013). They were recruited during a visit to their doctor to confirm that they were not experiencing symptom flare up at the time of the study. Data were collected from 2013 to 2016. The inclusion criteria were as follow: aged between 18 and 65 years; diagnosed according to medical diagnostic criteria with SLE, SS, or SSC; to be literate; have no severe psychiatric disorders; and not have undergone any corticosteroid treatment for a minimum of one year before the study. Healthy women met the same inclusion criteria but did not present physical illness. They were recruited through posters in public institutions, newspapers, and the local radio. Because of their potentially negative effect on cortisol levels (Williams et al., 2004), the exclusion criteria were: obesity, clinical diagnosis of depression or anxiety, personality disorders, and substance use (i.e., amphetamines, methadone, barbiturates, or muscle relaxants). This information was obtained through a semi-structured interview conducted when the women called to participate in the study. The study focused on these diseases because they are much more prevalent in women in both hospitals. In addition, they are systemic and autoimmune diseases with common genetics. There is a considerable amount of literature explaining common autoimmune disease manifestations and possible therapeutic targets in adults (Kochi, 2016) as well as children (Li et al., 2015).

All participants read the study information sheet and gave their signed informed consent to participate in the study, which was approved by the Ethics Committee and conducted in accordance with the Declaration of Helsinki (Mehring, 2015). This study was not preregistered.

2.2 | Procedure

A brief telephone interview was conducted with each woman to ensure they met the criteria to be included either in the healthy group or the autoimmune disease group.

The laboratory task was scheduled according to the diurnal cortisol curve, between 15:00–18:00 h, when salivary cortisol levels are more stable in the Spanish population (Santos-Ruiz et al., 2010). Once they agreed to take part, participants were informed on the study. They then read and signed the informed consent form, which had been approved by the ethics committees of both hospitals and was in accordance with Helsinki Declaration recommendations. The procedure was similar to that of the study of Montero-López et al. (2016). The processes of stressful situation exposure and data collection then began. Figure 1 shows the three-dimensional virtual audience projected.

Participants were instructed how to collect salivary cortisol samples during the day. They were then given a kit containing 5 Salivette® tubes and a schedule with numbered samples to collect at regular, specific times as well as a record sheet to write down the date and time of each collected sample. In addition, to become familiar with the experience of collecting saliva, the participants collected the samples the one day after performing the stress task in the laboratory. We made sure on that same day in the laboratory that they had understood how to follow the entire collection procedure and we answered their questions. We instructed the participants to avoid eating, chewing, having sweets or candy, and drinking (except water). Moreover, they were not allowed to smoke during the half hour preceding each sample collection. All participants were tested at the lab between Monday and Thursday, and they collected the saliva samples the next day. The salivary cortisol samples were thus collected on weekdays, not at the weekend. They were told to introduce and soak the cotton swab for 1 minute. These instructions applied to the collection of salivary cortisol samples at the lab and at home. For example, they self-collected the salivary samples at the lab during the stressful task to ensure they were capable of collecting the sample correctly and that the saliva did not have red bloods cells or any other contaminant. To ensure their participation, they received a financial incentive of € 20, which was given to them on the day they delivered the samples.

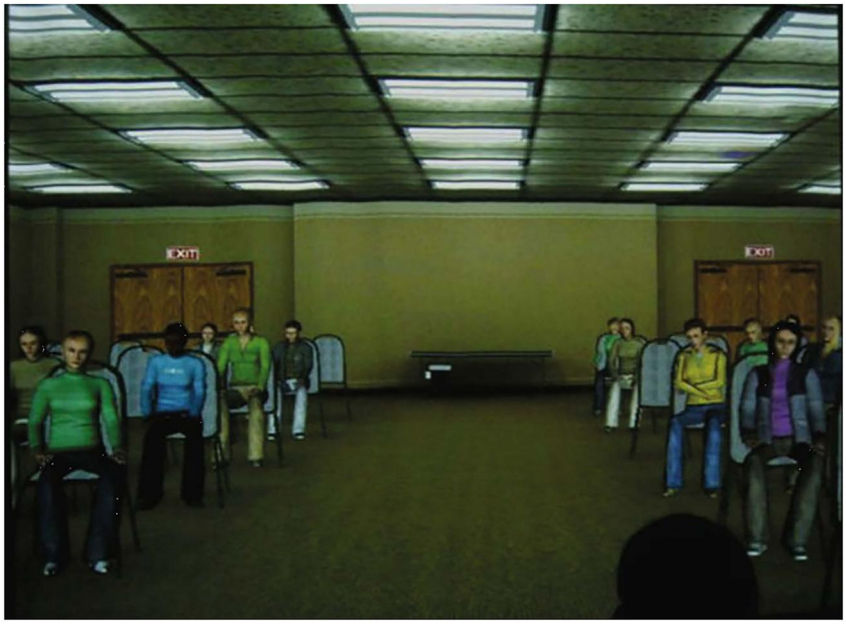


FIGURE 1 Three-dimensional virtual audience projected onto a large screen.

The salivary cortisol samples of two patients with SS could not be measured because there was not enough saliva to analyse the cortisol levels over one day. Two salivary cortisol samples with outlier values of TSST-VR in the SLE group were excluded from the analysis.

2.3 | Measures

2.3.1 | Demographics and psychological measures

Semi-structured interviews. The objective was to collect socio-demographic data, as well as data on daily life, sleep habits, medication, and history of psychological or psychiatric treatment. In the case of autoimmune patients, this information was completed with their medical histories.

Stress Vulnerability Inventory (SVI). The SVI (Beech et al., 1986; Robles-Ortega et al., 2006) measures the propensity to be affected by perceived stress.

Perceived Stress Scale (PSS). The PSS (Cohen et al., 1983; Remor & Carrobles, 2001) evaluates levels of perceived stress and the degree to which people find that their lives are unpredictable, uncontrollable, or overwhelming (aspects that contribute to stress) over the past 7 days, including the day the scale is administered.

Symptom Checklist-90-R (SCL-90-R). The SCL-90-R (De las Cuevas et al., 1991; Derogatis, 1994) consists of nine specific dimensions (somatisation, obsessive-compulsive, interpersonal sensitivity, depression, anxiety, hostility, phobic anxiety, paranoid ideation and psychoticism) and measures psychopathological symptoms.

Short Form Health Survey (SF-36). The SF-36 (Ware and Sherbourne (1992); Alonso et al., 1995) consists of 36 items that measure eight dimensions (physical functioning, social functioning, physical role, bodily pain, mental health, social role, vitality, and general health perception).

Slater-Usoh-Steed Questionnaire (SUS). The SUS (Usoh et al., 2000) assesses the sense of presence in virtual environments.

Trier Social Stress Test adapted to virtual reality (TSST-VR). The TSST-VR (Montero-López et al., 2016) is a psychosocial laboratory stress task that activates the SNS and the HPA axes, both of which are involved in the stress response. Subjects are given 5 min to prepare a 5-min speech in which they describe their strengths and weaknesses to a virtual audience, and then perform an arithmetic task for 5 min. Figure 2 shows the stress induction protocol employed.

2.3.2 | Stress measures

Sympathetic reactivity measurements: Skin conductance. Skin conductance was measured in the palm of the hand using two standard size Ag/AgCl electrodes connected to a BIOPAC MP150WSW. This system is highly sensitive to psychophysiological activation signals. When the electrodes were placed on the palm of the hand, the recording was checked on the monitor to verify that it was correct, that is, that the signal was clear and without artefacts. Although patients with autoimmune disease may present clinical manifestations of Raynaud's syndrome or sclerodactyly, all recordings in our study sample were properly. The data were recorded before the TSST-VR (baseline measurement at 5 min) and during three virtual scenario visualisation periods: anticipatory stress (5 min), speech (5 min), and arithmetic task (5 min).

HPA axis measurements: Salivary cortisol. Five salivary cortisol samples were obtained using a Salivette® (Sarstedt, Nümbrecht, Germany, Ref. 51.1534). These were analysed at the San Cecilio Clinical Hospital in Granada using the electrochemiluminescene immunoassay "ECLIA" method. This method was designed to be used in Roche Elecys 1010/2010 automated analysers with the Elecys MODULAR ANALYTICS E170 module. The first sample was collected at 4 PM, 30 min after arriving at the laboratory (basal cortisol = C1). Then, after giving the TSST-VR instructions, a second sample was obtained (pre-exposure cortisol = C2). Subsequently, the third sample (post-exposure cortisol = C3) was collected once the TSST-VR was completed. The fourth and fifth samples were obtained ten minutes (C4) and twenty minutes (C5) after finalising the TSST-VR, respectively. Basal cortisol sample (C1) was excluded of analyses because its levels do not report variations in the response to stressor. However, the cortisol sample C2 was used as a pre-exposure measure as its levels reflect the HPA axis activity around 5–8 min prior to the introduction to the stress task (Balodis et al., 2010).

To assess diurnal cortisol levels over one day and in a different situation to laboratory-induced stress, each participant collected five

<table>
<tr><th>Initial assessment</th><th colspan="5">Stress period</th><th colspan="4">Final assessment</th></tr>
<tr><td rowspan="2">Interview
SVI
PSS
C1
SF-36</td><td rowspan="2"></td><td colspan="4">TSST-VR</td><td rowspan="2"></td><td rowspan="2"></td><td rowspan="2"></td><td rowspan="2">SUS
SCL-90-R</td></tr>
<tr><td>Psychophysiological adaptation to VR (3')

SC</td><td>Anticipatory Stress (5')

SC</td><td>Speech delivery(5')

SC</td><td>Arithmetic task (5')</td></tr>
<tr><td></td><td>C2</td><td></td><td></td><td></td><td></td><td>C3</td><td>C4</td><td>C5</td><td></td></tr>
</table>

FIGURE 2 Diagram of the TSST-VR protocol. C1, basal cortisol; C2, pre-exposure cortisol; C3, post-exposure cortisol; C4, cortisol measured 10 min after exposure to the stressor; C5, cortisol measured 20 min after exposure to the stressor; PSS, Perceived Stress Scale; SC, skin conductance; SCL-90-R, Symptom Checklist; SF-36, Short Form Health Survey; SCL-90-R, Symptom Checklist-90-R; SUS, virtual presence questionnaire; SVI, Stress Vulnerability Inventory; TSST-VR, Trier Social Stress adapted to virtual reality.

salivary cortisol samples in a day. The first sample (CD1) was collected 30 min after waking up (while fasting), and the remainder of the samples were collected every 4 hours (CD2, CD3, CD4 and CD5) throughout the day until bedtime. First sample of diurnal cortisol was excluded of analyses since previous studies have demonstrated that the cortisol awakening response (CAR) and cortisol levels the rest of the day are independent markers of HPA axis activity (Stalder et al., 2016).

2.4 | Data analysis

Several ANOVAs were conducted to control for differences between four groups regarding socio-demographic variables (age and education level) and psychological variables.

To determine SNS activation differences between the four groups, we tested for possible interaction between conductance during the four TSST-VR periods (baseline line, anticipatory stress, speech and arithmetic task) and the four groups, using a 4 × 4 mixed ANOVA.

We verified the assumptions of normality and homogeneity of variance using Kolmogorov-Smirnov and Levene tests. Since cortisol measurements did not show a normal distribution, we followed the analysis recommendations of Stalder and Kirschbaum (2012), and we performed log transformation and ANOVAs with the transformed data for cortisol samples. To detect any HPA axis activity differences between HW, SS, SLE, and SSc during the TSST-VR at the time the cortisol samples were collected (C2, C3, C4 and C5), we performed a 4 × 4 mixed ANOVA.

To test whether there were any differences between the four groups regarding diurnal cortisol secretion, the cortisol levels collected four times in the day were compared (CD2, CD3, CD4 and CD5) using a 4 × 4 mixed ANOVA.

The Greenhouse-Greisser correction was applied to all repeated-measure analyses. For the analyses in which interaction was observed, we performed a within-group comparison using the Bonferroni statistic to determine differences between the times assessed for each group individually.

Moreover, we calculated the area under the curve of cortisol levels using the trapezoid formula (Pruessner et al., 2003). For salivary cortisol measurements during the TSST-VR, we calculated the total response with respect to the ground (AUCg) to obtain patterns of change during the observation period and total hormone production. Second, we estimated the area under the curve with respect to the increase (AUCi) in order to determine the increase and the overall intensity of cortisol level changes and cortisol response sensitivity during the TSST-VR. Both indexes were calculated with samples C2 to C5. Subsequently, we performed two ANOVAs to test for differences between the four groups where the independent variable had four levels (HW, SS, SLE and SSc) and the dependent variables were the AUCg and AUCi.

We also calculated with CD1 to CD5 samples, the AUCg for cortisol collected over one day and obtained total secreted cortisol production. Then, we performed various ANOVAs to identify any differences between the four groups.

All data were analysed using IBM® SPSS® version 21 (Armonk, NY).

3 | RESULTS

3.1 | Sociodemographic and psychological data

Table 1 presents the sociodemographic, psychological and disease data for each group. We found statistically significant differences between sociodemographic variables such as age and education, in somatisation and depression, and in quality-of-life subscales. There were no group differences in the other sociodemographic and psychological variables, including the sense of presence in a virtual environment in the TSST-VR.

3.2 | Sympathetic reactivity: Skin conductance

The mixed ANOVA showed no interaction between groups and different times (baseline line, anticipatory stress, speech, and arithmetic task), indicating that the four groups presented a similar sympathetic response to a stressful situation.

3.3 | Activity of the HPA axis: Salivary cortisol levels during the TSST-VR

After exposure to the virtual reality stress task, the results showed a time × group interaction effect [F (3,89) = 3.226; p = 0.003] in salivary cortisol secretion (Figure 3). The SSc group presented a similar activation pattern to that of the HW group when exposed to the laboratory stressor, whereas the HPA axis response in the SS and SLE groups was not activated.

Within-subject analyses showed HW differences between C2 and C4 (p = 0.008), C2 and C5 (p < 0.001), C3 and C4 (p < 0.001), and C3 and C5 (p < 0.001), and C4 and C5 (p = 0.005) Within-subject differences were also observed in SLE, at times C3 and C5 (p = 0.002). The SS and SSc groups did not present cortisol secretion differences at the different times, which could mean that the HPA axis response was not activated.

With respect to total cortisol production (AUCg) during the TSST-VR, the analyses showed a statistically significant differences [F (3,87) = 6.295; p = 0.001], between the HW and SSc groups (p < 0.000) and it was higher in women with SSc. There were no differences in AUCi levels between the four groups.

TABLE 1 Means, standard deviations and ANOVAs for the sociodemographic, psychological and disease data in four comparison groups.

Variables	HW (n = 37) M ± SD	SS (n = 21) M ± SD	SLE (n = 21) M ± SD	SSc(n = 18) M ± SD	p	Post- hoc
1. Sociodemographics:						
Age (years)	36.31 ± 9.31	48.95 ± 10.35	40.38 ± 7.46	50.28 ± 10.30	0.001*	HW, SLE≠SS, SSc
Education (years)	17.19 ± 2.92	14.86 ± 3.76	16.05 ± 2.52	12.94 ± 4.12	0.001**	HW≠SSc
Tobacco (%)	40.62%	14.28%	38.09%	5.55%	0.066	-
2. SCL-90-R:						
Somatisation	51.50 ± 11.53	64.05 ± 10.52	56.62 ± 7.48	58.29 ± 11.11	0.001**	HW≠SS, SLE, SSc
Obsessive/Compulsive	53.13 ± 12.61	61.16 ± 12.64	58.86 ± 7.92	61.35 ± 8.71	0.028	-
Interpersonal sensitivity	50.78 ± 11.7	58.26 ± 12.89	54.62 ± 13.54	56.47 ± 10.60	0.168	-
Depression	48.34 ± 9.59	57.84 ± 13.76	54.29 ± 10.26	57 ± 11.68	0.013*	HW≠SS
Anxiety	50.88 ± 10.07	58.26 ± 11.37	55.19 ± 10.40	53.24 ± 9.78	0.104	-
Hostility	48 ± 9.01	54.53 ± 10.92	52.76 ± 9.61	51.47 ± 10.93	0.122	-
Phobic anxiety	41.78 ± 11.68	51.16 ± 16.03	46.14 ± 12.70	48.94 ± 14.18	0.087	-
Paranoia	48.09 ± 12.70	55.47 ± 11.95	51.19 ± 13	50.41 ± 10.35	0.230	-
Psychoticism	45.63 ± 12.02	55.21 ± 14.05	53.76 ± 11.84	54.29 ± 10.28	0.016*	HW≠SS
3. Stress:						-
Stress vulnerability inventory	6.06 ± 4.08	11.14 ± 5.95	9.14 ± 5.48	9.08 ± 4.88	0.005*	HW≠SS
Perceived stress scale	21.69 ± 7.65	27 ± 10.48	27.19 ± 8.96	26.22 ± 10.84	0.098	-
4. SF-36:						
Physical functioning	94.50 ± 19.40	67.14 ± 29.85	77.38 ± 23.69	62.65 ± 26.64	0.001**	HW≠SS, SSc
Social functioning	83.33 ± 22.82	60.71 ± 27.18	64.88 ± 28.12	58.89 ± 25.68	0.003**	HW≠SS, SSc
Physical role	91.67 ± 21.10	48.81 ± 49.03	47.62 ± 43.95	36.76 ± 46.03	0.001**	HW≠SS. SLE, SSc
Bodily pain	75.86 ± 22.63	42.10 ± 23.35	50.52 ± 27.96	50.47 ± 29.32	0.001**	HW≠SS, SLE, SSc
General mental health	69.73 ± 11.77	53.90 ± 22.50	57.14 ± 19.27	56.94 ± 20.61	0.011*	HW≠SS
Social role	62.22 ± 40.80	49.21 ± 46.69	50.79 ± 49.01	52.94 ± 47.22	0.728	-
Vitality	60.50 ± 16.15	43.10 ± 24.41	47.62 ± 19.40	38.53 ± 24.92	0.003*	HW≠SS, SSc
Perception of general health	75.33 ± 15.47	39.48 ± 21.92	50.33 ± 19.27	38.88 ± 18.41	0.001**	HW≠SS, SLE, SSc
5. Years of disease	-	6.75 ± 4.91	9.95 ± 8.48	8.647 ± 5.58	0.304	-

Note: Data are expressed as mean and standard deviations.

Abbreviations: HW, healthy women; SLE, women with lupus; SS, women with Sjögren's syndrome; SSc, women with systemic sclerosis.

**$p \leq 0.01$; *$p \leq 0.05$.

3.4 | Cortisol levels during the day

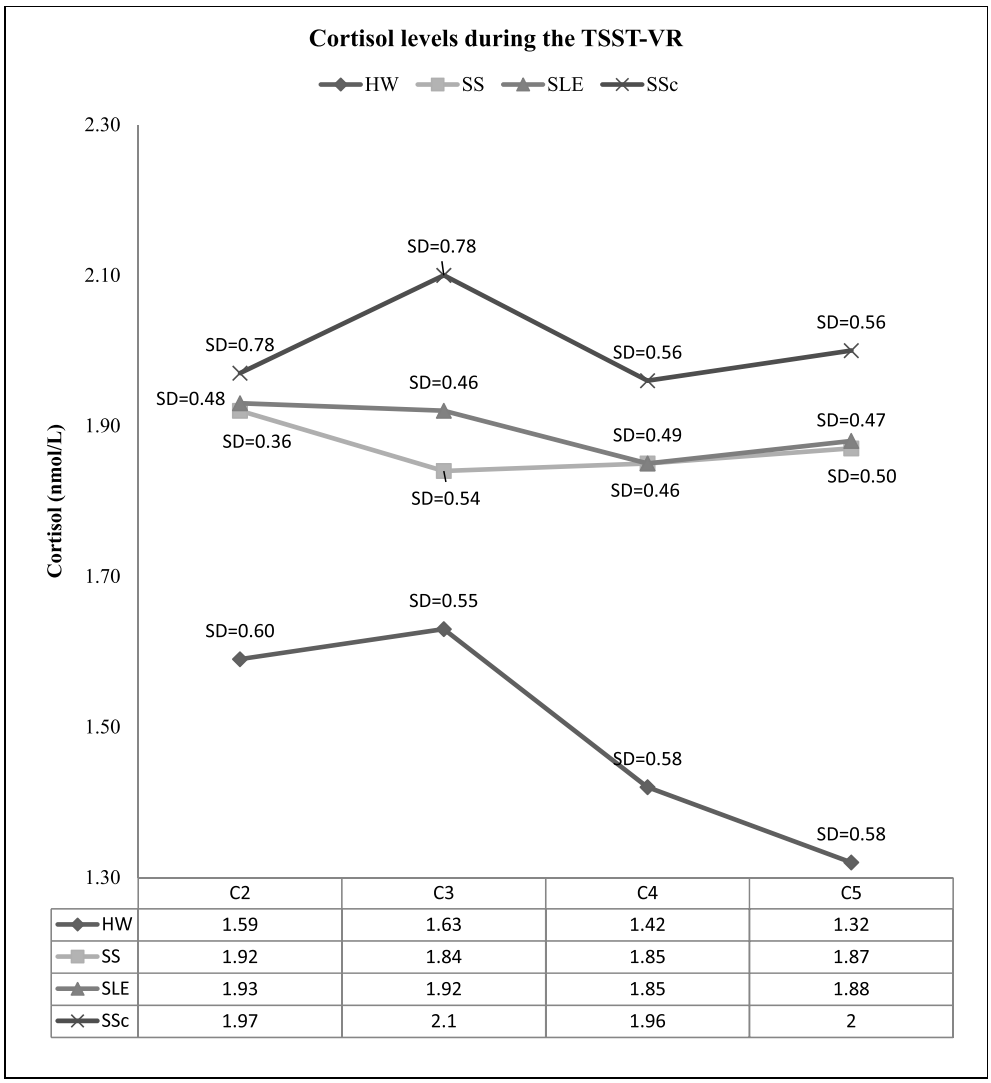
The results showed that there was not a time × group interaction between the four groups and the different time of the day.

The results showed a statistically significant differences in the cortisol AUCg collected one day [F(3,68) = 7.738; $p < 0.001$]. Differences were observed between healthy women and patients with SLE ($p = 0.004$) as well as with SSc ($p = 0.001$): women with SLE and SSc presented higher total hormone production than healthy women.

We found no differences between the three autoimmune diseases regarding cortisol secretion over one day.

4 | DISCUSSION

The present study is part of a line of research that is seeking to identify the mechanisms underlying the effect of psychological stress in autoimmune diseases. Hence, the aim of this study was to determine the activity of the axes of response to stress in patients with systemic autoimmune disease (SLE, SS and SSc) and in healthy women during a virtual reality psychosocial stress task. To this end, we analysed skin conductance and salivary cortisol in all subjects as measures of acute stress triggered by the stressor in order to assess SNS activity and HPA axis activity, respectively. The following day, we analysed HPA



	C2	C3	C4	C5
HW	1.59	1.63	1.42	1.32
SS	1.92	1.84	1.85	1.87
SLE	1.93	1.92	1.85	1.88
SSc	1.97	2.1	1.96	2

FIGURE 3 Cortisol levels during the TSST-VR. C2, pre-exposure cortisol; C3, post-exposure cortisol; C4, post-exposure cortisol +10 min; C5, post-exposure cortisol +20 min; HW, healthy women; SS, women with Sjögren's syndrome; SLE, women with systemic lupus erythematosus; SSc, women with Systemic Sclerosis.

axis activity through the pattern of salivary cortisol collected over one day as a measure of diurnal cortisol concentrations. In addition, we administered questionnaires to all participants to assess their psychological stress, quality of life, and psychopathology.

We obtained the expected results regarding psychopathological and health-related quality of life variables when comparing people with disease and healthy people. Patients with autoimmune disease presented worse scores for the SCL-90-R *somatisation* variable and the SF-36 *bodily pain*, *general health perceptions* and *physical role* variables than healthy subjects. Specifically, patients with Sjögren's syndrome obtained worse scores than healthy women on *mental health*, *depression*, *psychoticism* and *vulnerability to stress*. These results are in line with those reported in other studies where patients with Sjögren's syndrome presented a higher rate of psychiatric disorders (Shelomkova et al., 2013). In addition, patients with Sjögren's syndrome and those with systemic sclerosis differed from healthy women regarding the quality-of-life variables of *physical functioning*, *social functioning*, and *vitality*. These results could be attributed to the symptoms of Sjögren's syndrome and systemic sclerosis, or to the age of the members of these two groups, since they were older than the healthy women and the subjects with SLE. In this respect, Segal et al. (2009) assessed the health-related quality of life of patients with primary Sjögren's syndrome compared to healthy controls. The authors concluded that the symptoms experienced by patients with SS were related to the disease and could not be attributed to natural ageing processes. Consequently, the fact that the women in these two groups were older than the healthy women and women with SLE does not constitute a study limitation.

Patients with autoimmune disease showed acute levels of perceived stress, whereas healthy women presented normal levels. The differences, however, were not statistically significant. We did find significant differences though between healthy women and women with SS in the stress vulnerability inventory, which mainly identifies perception of stress-related physical symptoms. The absence of

satisfactory adaptive coping strategies presented by patients with SS (Karaiskos et al., 2009) could explain why we found that the SS group showed greater vulnerability to stress than the healthy women group.

Regarding SNS activation in a situation of acute stress, our findings are in line with those reported in a literature review by Finan and Zautra (2013) on patients with RA. Indeed, we did not observe any differences in SNS activation measured through skin conductance between women with systemic autoimmune disease and healthy women, a question that is under debate in RA studies.

Nevertheless, we did find HPA axis activity differences between healthy women and patients with autoimmune disease in the situation of acute laboratory stress. Specifically, healthy women showed the traditional cortisol secretion pattern during the TSST-VR, consisting of an increase in cortisol secretion 15 min after exposure to stress, accompanied by subsequent recovery. We also observed this same pattern in patients with SSc, but with higher salivary cortisol levels than in healthy women and a rise in cortisol in the recovery period. The other two groups with disease did not show an increase in cortisol secretion because of exposure to stress, and the HPA axis remained inactive. This absence of HPA axis activity during acute stress in women with SS and SLE may be associated with exacerbation of autoimmune disease due to daily stress, as suggested by the hypothesis of Munck et al. (1984), according to which the immune response triggered by the onset of the stressor is not suppressed. These results are consistent with those of other studies on patients with autoimmune disease and healthy controls, which found differences in immune responses in autoimmune patients (Delevaux et al., 2013; Straub, 2014; Straub et al., 2010).

Furthermore, they cannot be extrapolated to people with SSc since the latter showed an HPA activation similar to that of healthy people. The stress response differences observed between patients with SLE and Sjögren's syndrome compared to patients with systemic sclerosis may be due to pathogenesis differences between these diseases. Although all three have an autoimmune component, the inflammatory component plays a less important role in systemic sclerosis, where fibrosis predominates over inflammation (Distler et al., 2017; Thomas et al., 2017). In fact, the role of glucocorticoids in SSc treatment is minimal, since doses exceeding 15 mg per day should be avoided because they can trigger a renal crisis. In this regard, research has been conducted on the effect of anti-fibrotic agents (Iudici et al., 2013; Kowal-Bielecka et al., 2009).

Thus, not only did the groups of patients with systemic autoimmune disease present a different HPA axis response with respect to the HW group, but the axis activity pattern also differed between these three groups in acute stress situations. In other words, according to our results, the mechanisms underlying the exacerbation of symptoms in autoimmune diseases in response to psychological stress are not generalisable to all systemic autoimmune diseases.

With respect to diurnal cortisol levels over one day, we found no differences between healthy women and patients with autoimmune diseases. According to neuroendocrine immunology, the HPA and SNS axes are synchronised through circadian activation in the early hours of the morning and inhibited at midnight (Straub, 2014).

These findings indicate that each autoimmune disease presents different cortisol fluctuations during the day, which could underlie differences in HPA axis activation in response to a psychosocial stressor compared to healthy individuals, increasing the likelihood of autoimmunity.

The results of this study should be considered in the light of the limitations described next. Salivary cortisol levels are influenced by each person's circadian rhythms (Pruessner et al., 2003). In addition, the time of awakening should be recorded because it influences diurnal cortisol levels (Stalder et al., 2016, 2022). Therefore, including other measures such as α-amylase or the erythrocyte sedimentation rate could provide further information on the response to stress and diurnal cortisol patterns in patients with autoimmune disease.

Lastly, it would be of interest in future studies to increase the sample size and include additional groups of autoimmune diseases, in order to further explore the behaviour of axes of response to stress.

To conclude, our findings confirm that the HPA axis in patients with autoimmune disease functions differently from that of healthy people, generating different patterns of cortisol secretion in response to stress according to the autoimmune disease in question: SLE, SS, or SSc. The reason may lie in the specific pathogenesis of each disease. A greater understanding of the explanatory mechanisms of deterioration of autoimmune patient symptoms due to psychological stress could help to improve the treatment of these patients, reduce damage, and enhance their health-related quality of life.

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CONFLICT OF INTEREST STATEMENT

Non-declared.

DATA AVAILABILITY STATEMENT

The data are available on request from the first author. The data are not publicly available due to privacy or ethical restrictions.

ETHICS STATEMENT

This study was approved by the Human Research Ethics Committee of the University of Granada (PSI2010-15780).

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