

## Psychophysiological responses to acute stress in two groups of healthy women differing in fitness

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This study examined how the degree of fitness affects several psychophysiological responses to stress in elite sportswomen and physically active women. Trait anxiety and mood were evaluated, and salivary testosterone (Tsal) and cortisol (Csal) concentrations determined before and after a maximal ergometry. Afterwards, subjects carried out the Stroop task, where heart rate (HR) and skin conductance level (SCL) were continuously recorded. At baseline, elite sportswomen had worse mood, and lower cardiovascular basal measures and testosterone:cortisol ratio. In the Stroop task, they showed higher SCL reactivity and worse recovery with respect to the baseline values than physically active women. There was a positive relationship between Tsal and HR as well as between negative mood and SCL, and a negative one between negative mood and HR. In women, the degree of fitness moderates the electrodermal responses to acute stress, a specific pattern of relationship among different psychophysiological variables being found.

*Respuestas psicofisiológicas a estresores de laboratorio en mujeres con diferente condición física.* El objetivo de este estudio es analizar cómo la condición física modula diferentes respuestas psicofisiológicas al estrés en deportistas de élite y mujeres físicamente activas. Para ello, se evaluó la ansiedad y el estado de ánimo y se determinaron los niveles hormonales de testosterona y cortisol antes y después de la realización de una cicloergometría máxima. Tras ello, los sujetos realizaron el Test de Stroop, en el que se registró continuamente la frecuencia cardíaca y la actividad electrodérmica. Antes de las pruebas las deportistas de élite tenían peor estado de ánimo, menores valores cardiovasculares basales y menor ratio testosterona:cortisol que las físicamente activas. Además, mostraron mayor reactividad y peor recuperación electrodérmica en el Test de Stroop. La frecuencia cardíaca correlacionó en positivo con la testosterona basal y con el estado de ánimo negativo. Por tanto, la condición física modula las respuestas electrodérmicas al estrés agudo en mujeres.

A number of studies over recent years have assessed physiological responses to mental stress during exercise recovery. Most of them have been carried out in men, and aimed to evaluate whether acute bouts of physical exercise acts as a buffer of these psychophysiological responses (Roy and Steptoe, 1991; Steptoe, Kearsley and Walters, 1993; West, Brownley and Light, 1998). Other studies have analyzed the psychological, endocrine or autonomic responses to physical and mental laboratory stressors after comparing trained and sedentary people to determine whether physical fitness moderates the psychophysiological reactivity to stress. Cardiovascular responses to different stressors have been studied in sports persons, where physical activity buffers these responses and intervenes in maintaining health (Szabó et al., 1994; Guirado et al., 1995; Moya-Albiol, Salvador, González-Bono, Martínez-Sanchís and Costa, 2001a). Only in two studies have the effects of physi-

cal fitness on the psychophysiological responses to mental stress after acute physical exercise been analyzed in women. In one case, the level of physical activity of the subjects did not attenuate the physiological and subjective responses to a mental task. This was shown by the lack of differences between high and low physically active women who carried out a modified version of the Stroop Color Word Task after a cycle ergometer test at 70% of the subject's age-predicted maximal heart rate (HR) (Duda, Sedlock, Melby and Thaman, 1988). The other study did not report significant differences in the psychophysiological reactivity to mental tasks after comparing physically active to inactive subjects (Roth, 1989). Nevertheless, no studies have compared elite sportswomen to physically active women, this latter group having a profile of habitually practicing moderate physical activity, the kind of exercise which proportions the highest benefits to health (Weyerer and Kupfer, 1994).

The first aim of this study was to determine whether the differences in practicing exercise and the underlying different level of fitness could also affect the response to physical and psychological stress in women, after comparing a group of elite sportswomen with another group of physically active women. Our general prognostic was that physically active subjects had higher psychophysiological response to the stressors. Based on previous data (Häk-

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Fecha recepción: 7-11-02 • Fecha aceptación: 7-4-03

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kinen and Pakarinen, 1993; Wheeler et al., 1994) and in a previous study carried out in elite sportsmen and physically active men (Moya-Albiol et al., 2001b), we hypothesized an increase in salivary testosterone (Tsal) in response to the ergometry for all subjects. When comparing elite sportswomen and physically active subjects, we expected lower increases for salivary cortisol (Csal) and higher response for the Tsal/Csal ratio in the former. This is due to the fact that elite sportsmen have a better adaptation to the maximal ergometry measured by the T/C ratio, and as they are used to this kind of physical effort the Csal increases are lower than in other subjects. Due to the different level of fitness, we also hypothesized lower autonomic activation and better recovery from mental stress in elite sportswomen than in physically active women. To afford a better understanding of the underlying mechanisms to stress response, another aim of the study was to verify the existence of relationships between different psychophysiological variables. We expected an association between Tsal and HR as has been found in men (Girdler, Jamner and Shapiro, 1997; Moya-Albiol et al., 2001a; 2001b), as well as between HR and mood, following previous results (Carrillo et al., 2001). For these purposes, Tsal and Csal levels in elite sportswomen and physically active women were analysed before and after a maximal cycle ergometer test as were HR and SCL changes in response to the Stroop task carried out after the ergometry. Together with type-A behaviour pattern (Pallarés and Rosel, 2001) anxiety and mood are the two psychological aspects which were most likely to affect these responses. Thus, they were controlled since we have found that both variables are related to autonomic responses to laboratory tasks in women (Carrillo et al., 2001).

#### Method

##### *Subjects*

The final sample was composed of 9 elite professional sportswomen and 11 young physically active women, whose characteristics are shown in Table 1. The sportswomen who were doping-controlled, trained between 10-15 hours a week, and were recruited by means of their coaches. The other group was selected from a sample of healthy university students recruited by teachers. The initial pool was composed of 50 subjects, those who had the highest estimated maximum oxygen uptake ( $VO_2$  max) being selected. The estimated  $VO_2$  max was calculated from the Physical Activity Index (PA-R), weight, height, gender and age, following the procedure indicated by Jackson et al. (1990). Their level of physical activity was inside the range proposed by public health guidelines (USA & UK) for health benefits. Both groups were quite different, the control group not being physically active enough to make it similar to the elite sportswomen. Subjects did not use medication, were non smokers, regularly menstruating, and did not take contraceptives. All participants gave an informed consent approved by the Local Ethics Committee.

##### *Saliva Collection and Hormonal Determination*

Saliva was directly collected from mouth to tube (Unitek<sup>®</sup>). Subjects were informed about the necessity of following the instructions for saliva sampling, in order to obtain valuable data. Samples were centrifuged (5000 rpm,  $15 \pm 2$  °C) and frozen at  $-20$  °C until determination by radioimmunoassay at our laboratory

(Central Research Unit, Faculty of Medicine, University of Valencia, Spain). Samples from each subject were run in duplicate in the same assay. More details about hormonal determination have been previously described elsewhere (González-Bono, Salvador, Serano and Ricarte, 1999).

##### *Procedure*

Each subject of both groups participated in one single session that was carried out at the Sports Medicine Centre (Cheste, Valencia, Spain), elite sportswomen being at the beginning of the sports season. The session lasted from 9.00 to 14.00 hours. Data were collected in different rooms isolated from noise, with constant temperature ( $22 \pm 2$  °C) and humidity ( $50 \pm 10\%$ ). After a period of habituation to the environment, the subject provided the first salivary sample and answered (9.00-9.30 h) a Spanish version of the POMS conveniently validated (Balaguer, Fuentes, Meliá, García-Merita and Pérez-Recio, 1993). Secondly, a medical interview, anthropometric measurements, resting HR (Kenz-ECG 302) and blood pressure (BP) (Speidel-Kellep) registers and administration of a Spanish version (TEA) of the State-Trait Anxiety Inventory (Spielberger, Gorsuch, Lushene, Vagg and Jacobs, 1983) were carried out (9.30-11.00 h). Anthropometric measures were obtained according to the Pollock and Jackson method (Pollock and Jackson, 1984). Afterwards, between 11.00-12.30 h approximately, each subject performed a maximal ergometer test until voluntary exhaustion, including measurements of several physiological parameters. After the test, the subject relaxed for a while before the collection of a second salivary sample and the completion of the S-STAI. Between 20 and 30 minutes later, the subject was conducted to another room where she carried out a modified version of the Stroop Color-Word Task (MacLeod, 1991) while HR and SCL were simultaneously measured (between 12.00-14.00 h, approximately). The recording was performed by means of a Coulbourn Modular Recorder System (model S16-12, PA, USA), and the Optical Pulse Coupler (S71-40) and the Tachometer (S77-26) were used for the acquisition and processing of the heart signal, respectively. The transducer was an IR-LED Phototransistor with a frequency of response oscillating between 0.5 to 10 Hz. A third module, the Skin Conductance Coupler (S71-22) was used for the transduction and registration of SCL. The subject had to stay quietly and relaxed seated in front of the computer where the task was presented and afterwards perform a 3-min practice with the electrodes fixed. After 10 min of relaxation, HR and SCL were measured for baseline (5 min), the task (5-6 min), and post-task (3 min).

##### *Data Reduction and Analyses*

One-way ANOVAs were performed to compare both groups in anthropometric, psychological, baseline physiological measures, and measures in the ergometry, as well as task performance. Repeated Measures ANOVAs with 'type of items' (numeric/non numeric) as within-subjects factor was computed to verify the 'Strop effect'.

For hormonal levels, repeated measures ANOVAs with 'Time' (Basal/Post-ergometry) as within-subjects factor, and 'Group' (elite sportswomen/physically active subjects) as between-subjects factor were carried out, with Greenhouse-Geisser adjustments for degree of freedom where appropriate. To measure the hormonal

responses, the difference between post-ergometry and basal levels was calculated, and one-way ANOVAs were performed to compare between groups.

In the case of HR and SCL, mean values for baseline, task, and post-task periods were obtained, using Acqknowledge software. With respect to autonomic measures, repeated measures ANOVAs with 'Period' (Baseline/Task/Post-task) as within-subjects factor, and 'Group' as between-subjects factor, using Greenhouse-Geisser adjustments for degree of freedom, were carried out. Reactivity was assessed via simple change scores (task minus baseline) while recovery was considered as the difference between post-task and baseline measures following previous recommendations (Linden, Earle, Gerin and Christenfeld, 1997). One way ANOVAs to compare the autonomic reactivity and recovery were performed.

ANCOVAs including body fat and body mass index (BMI) as covariate were carried out for hormonal and autonomic parameters. ANCOVAs with trait-anxiety and mood scores as covariates were also calculated.

As post-hoc tests, one-way or repeated measures ANOVAs/ANCOVAs were used depending on the cases.

Relationships between variables were analysed by Pearson or Spearman correlations. All the analyses were carried out by the SPSS 8.0 for Windows. Average values in the text are expressed as mean ± SEM. The alpha level was fixed at 0.05.

Results

Descriptive Characteristics and Baseline Psychophysiological Measures

There were no significant differences between groups in age, height, weight and BMI, but the body fat was significantly lower in elite sportswomen (F1,18= 16.87, p<0.001) (see Table 1).

No significant differences were found between elite sportswomen and physically active women in trait anxiety scores (19.78±3.01 and 13.54±2.06, respectively), but the former presented worse mood (Figure 1), with higher values in the total score (F1,16= 5.43, p<0.03) and in Anger (F1,16= 5.06, p<0.04) and Fatigue (F1,16= 7.35, p<0.02) scale scores.

With respect to baseline physiological measures (Table 2), basal HR and Systolic BP were lower in elite sportswomen than in physically active women (F1,18= 6.56, p<0.02 and F1,19= 6.98, p<0.016, respectively), although groups did not differ in Diastolic BP. There were no significant differences between groups in basal levels of Tsal and Csal. Nevertheless, basal levels of Tsal/Csal ratio were significantly lower in elite sportswomen (F1,15= 4.89, p<0.04).

	Elite sportswomen	Physically active women
Weight (Kg)	52.51±3.07	54.67±1.60
Height (m)	1.64±0.02	1.61±0.01
Age (years)	20.33±1.00	20.82±0.52
Fat (%)**	13.27±0.97	20.92±1.41
BMI (Kg/m <sup>2</sup> )	19.58±1.04	21.07±0.59

\*\*p<0.01

Response to the Ergometry

Measures in the ergometry

In the ergometry (Table 3), the VO<sub>2</sub> max and the maximal power output were higher in elite sportswomen than in physically active subjects (F1,19= 26.02, p<0.001 and F1,19= 21.35, p<0.001, respectively). There was no significant difference between either group in maximum HR, BP and the Maximal lactate (LA max).

State Anxiety and Hormonal Responses

No significant differences in State anxiety were found when elite sportswomen and physically active women were compared (14.22±1.79 and 16.18±2.19, respectively).

Concerning hormonal responses to the physical stressor, only a significant effect for 'time' was found for Tsal (F1,15= 7.10, p<0.02; P= 0.70), with an increasing pattern for both groups. For Csal, there was a significant effect for 'group' (F1,17= 5.59,

	Elite sportswomen	Physically active women
HR (bpm)*	51.25±4.84	63.64±2.20
Systolic BP (mmHg)*	103.89±2.17	111.82±2.05
Diastolic BP (mmHg)	61.67±0.83	63.18±1.39
Tsal (pmol/l)	34.27±6.49	43.26±3.35
Csal (nmol/l)	14.83±4.57	8.97±2.01
Tsal/Csal ratio*	0.003±0.001	0.008±0.002

\*p<0.05

	Elite sportswomen	Physically active subjects
Maximum HR (bpm)	186.89±1.99	187.27±3.35
Systolic BP (mmHg)	150.00±5.00	152.27±5.45
Diastolic BP (mmHg)	68.89±2.61	72.73±2.17
Maximal power (w)**	197.78±4.01	160.00±6.61
VO <sub>2</sub> max (ml/min/kg)**	37.52±0.55	29.65±1.31
LA max (nmol/l)	11.48±0.17	10.50±0.58

\*\*p<0.01

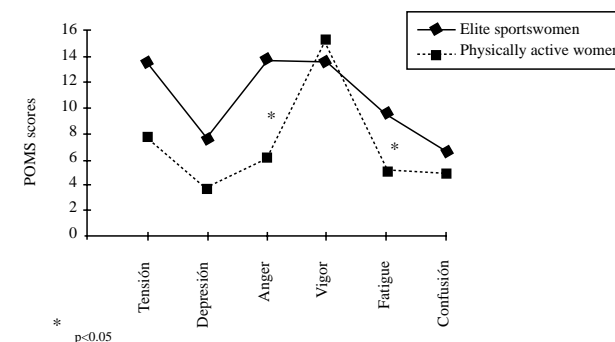
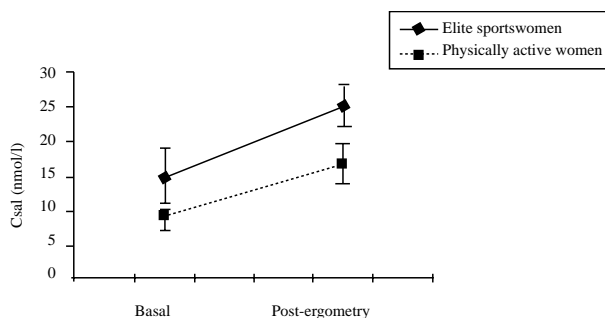


Figure 1. POMS scale scores for elite sportswomen and physically active subjects

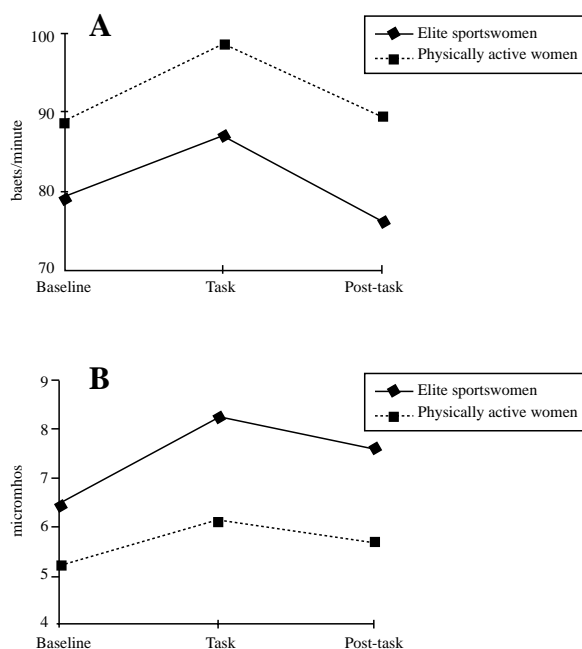
$p < 0.03$ ;  $P = 0.61$ ) and 'time' ( $F_{1,17} = 6.64$ ,  $p < 0.02$ ;  $P = 0.68$ ), with higher levels in elite sportswomen and an increasing pattern in both groups respect to the basal Csal (Figure 2). In addition, the groups did not differ significantly in Tsal/Csal ratio or hormonal changes. We repeated all hormonal analyses with body fat and BMI as covariate but no additional effects were found. After covarying trait anxiety, results did not change, but when covarying mood, no significant effects for 'group' were observed in the case of Csal.

#### Autonomic Responses and Performance in the Stroop Task

For HR (Figure 3), the factors 'group' ( $F_{1,15} = 5.90$ ,  $p < 0.03$ ) and 'period' ( $F_{1.5,22.48} = 18.13$ ,  $p < 0.001$ ;  $P = 1.00$ ) were significant, showing an HR increment when faced with the stressor ( $F_{1,16} = 15.43$ ,  $p < 0.001$ ;  $P = 0.96$ ), a post-task decrement ( $F_{1,16} = 49.11$ ,  $p < 0.001$ ;  $P = 1.00$ ) and no differences between baseline and post-task levels. The HR levels were significantly lower in elite sportswomen. Nevertheless, there were no significant differences between groups in HR reactivity and recovery.



**Figure 2.** Basal and post-ergometry levels of Csal for elite sportswomen and physically active subjects



**Figure 3.** Baseline, task, and post-task HR (A) and SCL (B) in the Stroop task for elite sportswomen and physically active subjects

In SCL (Figure 3), the factors 'group' ( $F_{1,16} = 4.55$ ,  $p < 0.05$ ;  $P = 0.52$ ) and 'period' ( $F_{1.46,23.41} = 47.69$ ,  $p < 0.001$ ;  $P = 1.00$ ), and the 'group x period' ( $F_{1.46,23.41} = 5.85$ ,  $p < 0.01$ ;  $P = 0.74$ ) interaction were significant. Post hoc analysis for 'period' showed a significant SCL increment when faced with the stressor ( $F_{1,17} = 49.40$ ,  $p < 0.001$ ;  $P = 1.00$ ) and a significant post-task decrement ( $F_{1,17} = 37.47$ ,  $p < 0.001$ ;  $P = 1.00$ ), whereas post-task SCL values were higher than the baseline ones ( $F_{1,17} = 21.71$ ,  $p < 0.001$ ;  $P = 0.99$ ). SCL was higher in task and post-task periods ( $F_{1,17} = 6.37$ ,  $p < 0.02$  and  $F_{1,17} = 4.95$ ,  $p < 0.04$ , respectively) in elite sportswomen than in physically active women. In addition, the former showed significantly higher SCL reactivity (task minus baseline levels) and recovery (post-task minus baseline levels) with respect to the baseline values ( $F_{1,17} = 7.99$ ,  $p < 0.01$  and  $F_{1,17} = 4.86$ ,  $p < 0.04$ , respectively).

For both HR and SCL, we repeated all analyses with body mass and BMI as covariate, but no additional effects were obtained. When considering both psychological dimensions (trait anxiety and mood) as covariates in the ANOVA, the effect of 'group' was not significant for SCL.

With regard to the Stroop Task, the 'Stroop effect' was significant for errors ( $F_{1,19} = 10.56$ ,  $p < 0.004$ ;  $P = 0.87$ ) and reaction times ( $F_{1,19} = 43.96$ ,  $p < 0.001$ ;  $P = 1.00$ ), but when the performance between groups was compared no significant differences were found.

#### Relationship between the Psychophysiological Variables.

For the total sample, basal Tsal was positively related to HR during task and post-task periods ( $r = 0.57$ ,  $p < 0.05$  and  $r = 0.56$ ,  $p < 0.05$ , respectively).

With regards to mood and autonomic responses, there was a negative correlation between the POMS-t and HR in the post-task period ( $r = -0.57$ ,  $p < 0.03$ , respectively), and a positive correlation between the score in this profile and SCL reactivity and recovery ( $r = 0.61$ ,  $p < 0.02$  and  $r = 0.83$ ,  $p < 0.001$ , respectively).

#### Discussion

In response to laboratory stressors SCL and Csal levels were significantly higher, and HR levels lower in elite sportswomen than in physically active subjects. Moreover, when the magnitude of the responses was compared, the groups differed in SCL reactivity and recovery, which were higher in the former group.

The lower body fat, basal HR, and systolic BP in elite sportswomen than in physically active women together with the significantly higher  $VO_2$  max and maximal power output in the ergometry confirm that both groups are very different, as the former has better physical fitness. As in other studies which have compared hormonal levels in trained women and sedentary controls (Tegelman et al., 1990; Tsai et al., 1991), no differences between the two groups in basal levels of Tsal and Csal have been found. Nevertheless, the basal Tsal/Csal ratio was lower in elite sportswomen. The glucolytic cost of the effort was equivalent in both groups since their post-exercise LA max values were similar. With respect to psychological dimensions, the groups did not differ in trait anxiety, and both had a positive mood according to the range proposed for sports people by Morgan, Costill, Flynn, Raglin and O'Connor (1988). Elite sportswomen showed worse mood measured by the total POMS score, and particularly higher anger and fatigue (see Figure 1).

In response to physical exercise, neither group differed in their state anxiety scores, but all subjects showed lower scores compared to the mean of the population in a basal situation. Even if the evidence for anxiolytic effects of maximal exercise is less convincing than moderate exercise, cycle ergometry could have reduced state anxiety, as has been found in several studies which have measured this variable after the practice of physical activity in a similar period of time (Raglin, Turner and Eksten, 1993; Raglin and Wilson, 1996). Contrary to our hypothesis both groups showed the same pattern of responses, with an increase in their basal levels of Tsal and Csal in response to the cycle ergometry. In contrast, the results in men found in a previous study (Moya-Albiol et al., 2001b), showed a slight diminution in Tsal and Csal levels in elite sportsmen and an increase in physically active men after the ergometry. Several investigations have indicated that women adapt differently from men to similar physical stress (Davis, Pate, Burgess and Slentz, 1987; Tegelman et al., 1990), reporting that increased intensity of physical activity could augment the concentrations of cortisol more in females than in males (Tsai et al., 1991). Also, differently from men and from our prognostic, both groups showed similar responses to the ergometry in the Tsal/Csal ratio, and as reported in another study no significant changes in this ratio have been found after the exercise in trained women (Tsai et al., 1991).

We have observed an increment in HR and SCL when both groups of subjects faced the stressor, and a post-task decrement. In addition, elite sportswomen showed lower HR as in another study with men (Boutcher, Nugent, McLaren and Weltman, 1998) and higher SCL during and after the task. In our previous study, elite sportsmen showed lower HR and SCL, which indicates that SCL responses to psychological stressors are different depending on the gender. Moreover, as in studies carried out in men (Dorheim et al., 1984; Claytor, Cox, Howley, Lawler and Lawler, 1988), we have not found differences between groups in cardiac reactivity and recovery, but elite sportswomen showed higher SCL reactivity and worse SCL recovery than physically active women. SCL has been used as one of the more widely employed indexes in evaluating psychological processes such as emotion, arousal and attention. Hence, elite sportswomen are more reactive to a psychological stressor and present a worse recuperation from stress (their post-task values are actually farther from their baseline ones) than physically active subjects. Our results support the idea that SCL could also be used as a good index to discern the responses to laboratory stressors in women who differ in the way in which practice physical activity.

Another important aspect of this study is the positive relationship obtained between Tsal and HR, due to the fact that high levels of androgens in women have been related to an increase in the risk of suffering cardiovascular disorders (Haffner, Katz, Stern and Dunn, 1988). Nevertheless, the majority of research which has studied this relationship has been carried out in men (Barrett-Connor

and Khaw, 1988; Haffner, Valdez, Mykkänen, Stern and Katz, 1994; Simon et al., 1997). For this reason, more studies are necessary to clarify how the hormonal levels may affect the HR responses to stress in different samples of active and sedentary women. Furthermore, women who had worse mood presented lower HR, and higher SCL responses. Research relating mood and electrophysiological responses to mental stressors is scarce, with most investigation based on depression. In response to another kind of stressor such as the speech task, the opposite pattern of relationship has been described, associating a worse mood with higher HR (Carrillo et al., 2001). For this reason, it is important to take into account the kind of stressful situation, as women seem to react differently when confronting social or mental stressors. Depressive patients have shown lower SCL levels than healthy people (Miquel, Fuentes, García-Merita and Rojo, 1999), which has been explained by inhibitory mechanisms in the information processing of the Central Nervous System (Boucsein, 1992). The association between SCL reactivity and recovery and negative mood throw more light on this question, suggesting an inhibition of SCL levels together with higher SCL responses in subjects with worse mood. The higher scores in the POMS in addition to the lower baseline HR and Tsal/Csal ratio, and to the higher SCL during the mental task in elite sportswomen indicate that at the beginning of the sports season they showed worse mood and hormonal balance. The lack of significant effects in Csal and SCL after covarying anxiety and mood indicate, as has been previously described (Carrillo et al., 2001) that psychological dimensions moderate the physiological responses to stress in women, a result which has not been found in men (Moya-Albiol et al., 2001b).

In summary, the results suggest that the degree of fitness moderates the electrodermal responses but not the hormonal (Tsal, Csal and Tsal/Csal ratio) and HR responses to physical and psychological laboratory stressors, specifically when elite sportswomen and physically active women are compared. This effect is strongly affected by the psychological dimensions such as trait anxiety and mood. In addition, a specific pattern of relationship among psychological, endocrine and autonomic responses has been found in women, and taking into account our previous study in men, we may conclude that gender is also an important variable to consider when the responses to stress are the aim of an investigation.

#### Acknowledgments

The authors wish to thank Ms. Miriam Phillips for the revision of the English text, and Dr. Carlos Sanchis for his collaboration. We also thank the General Sports Service of the Valencian Government for the use of facilities to carry out the laboratory sessions. This study was supported by grants no. SAF92-692 from the Spanish Committee for Scientific and Technical Research and no. 3732 from the Spanish Superior Council of Sports.

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