

PROLACTIN RESPONSES TO STRESS INDUCED BY A COMPETITIVE SWIMMING EFFORT

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Abstract. Purpose: The aim of the present study was to investigate the changes in prolactin (PRL) plasma concentrations induced by competitive swimming practice. **Methods:** Twenty-three males, 13 trained swimmers (experimental group) and 10 sedentary and healthy students (age-matched control group) took part in this investigation. The swimmers were assessed at three points: basal conditions, pre- and post-swimming competition (100 m freestyle), whereas subjects from the control group only undertook the basal trial. The variables analysed were: several body composition measures, anxiety level (STAI questionnaire), PRL and lactic acid concentrations. **Results:** No statistical differences were observed in PRL basal levels between groups. An evident PRL response to pre-competition psychological stress was observed in the experimental group, since the PRL plasma concentration rose from 4.02 ± 0.53 ng/ml (basal conditions) to 5.52 ± 0.53 ng/ml ($p \leq 0.05$). The PRL response to the competitive effort produced an important increase in its plasma concentration (10.07 ± 1.59 ng/ml), showed statistical differences from pre-competition ($p \leq 0.01$) and from basal conditions ($p \leq 0.001$). A significant rise in plasma lactate levels just at the end of the effort was found, although it did not correlate with PRL levels in the same situation. **Conclusion:** While we observed a remarkable response of PRL to psychological and physiological stress induced by a short term competitive effort in swimming, no changes in PRL basal levels were exhibited with swim training. More research is needed to clarify these findings.

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Introduction

The responses and adaptations of several hormones denominated “stress hormones” which are secreted from anterior pituitary gland, can give an integrated

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information about psychological and somatic stress that athletes can experience in a competitive effort.

Prolactin (PRL) is a polypeptidic hormone of a single chain with 198 aminoacids, structure very similar to the human growth hormone (hGH). Their principal biological actions are mamothropic and gonadothropic, although normally several external stimulus can improve the PRL secretion. Stress, from psychological or somatic origin, is one of this stimulus [1].

As for somatic stress induced by exercise, several authors have focused their attention in the study of PRL responses to a maximal efforts, under anaerobic energy source support. In this sense, rises over 100% from the PRL basal levels have been found after a progressive and maximal effort [7,22]. In other investigation, the response of PRL to an interval and exhaustive exercise was analysed in trained subjects. Just at the end of the effort PRL plasma concentrations rose 230% above of basal levels [17]. Also, a important increase in PRL plasma concentrations after an exhaustive exercise was found, although curiously the peak concentration was reached 60 min after exercise [14]. However, anaerobic efforts, of short duration and high intensity, like a 60 s of consecutive vertical jumps, leded to increase PRL plasma concentration, even though no statistical significance was found [5].

On the other hand, an important number of investigations to analyse PRL response related to aerobic exercises have been developed. In this sense, the alterations in PRL plasma concentration in long distance running (half marathon and marathon) have been analysed. In this studies, a significant increase in PRL concentration was observed at the end of exercise [25,30]. In spite of that, there are dates which offer some controversy to the indicated before, since after to examine PRL plasma concentrations in 11 male trained athletes before, during and at the end of a running race of 110 km, no variations were registered [15].

Although with some exceptions, everything points to aerobic and anaerobic exercises are presented like a sufficient stimulus to induce PRL secretion. In fact, after 60 min of continuous aerobic exercise and after 60 min of intermittent anaerobic exercise, the PRL plasma concentrations of nine subjects who participated in this investigation rose significantly above the basal levels [18].

Another objective to pursue in this type of investigations is the establishment of a minimal intensity of effort from which the PRL response and/or release of PRL occur. In the first investigations developed with this aim, the anaerobic threshold was established such as minimal intensity necessary for to induce the PRL response [9]. In the next investigations this intensity was located between 70 and 80% of



VO_{2max} , demonstrating that exercises of minor intensity are unable to alter PRL plasma levels, or even can lead to reduce them [12,21].

By the other hand, and related with stress from psychological source, there is no evidence that this type of stress would to stimulate the PRL response and to cause an increase of its plasma concentration. This is gathered from the few studies in which the responses of PRL to psychological stress related to competition have been evaluated [25,29].

After this precedents, the aims of the present investigation are: a) to determine the psychological stress (anxiety) generated by a highly demanding effort –an official swimming competition–, and assessing the PRL response to this event, and b) to determine the plasma PRL reactivity to the somatic stress induced by this maximal and short duration effort.

Materials and Methods

Thirteen national level male swimmers, all of whom were specialists in swimming distances of 100 and 200 m with an experience of at least 6 years in systematic training (experimental group) and ten healthy male students (age-matched control group) took part in this study. Informed written consent was obtained from all participants, and the project was approved by the ethical committee of the University of Granada. General and anthropometric characteristics of the subjects are presented in Table 1.

Table 1

Subject characteristics

| | Age (yr) | Height (cm) | Weight (kg) | BMI (kg/m ²) | Body fat (%) |
|--------------|----------------|-----------------|----------------|-----------------------------|-------------------|
| Experimental | 18.62 ±0.78 | 174.95 ±1.80 | 65.11 ±2.14 | 21.28 ±0.66 | 10.75 ±1.00 |
| Control | 19.10 ±0.82 | 171.55 ±2.24 | 74.07 ±4.19 | 25.09 ±1.15** | 21.59 ±1.93*** |

Data represent means ± SEM

p≤0.01, *p≤0.001 for Mann-Whitney test comparing experimental and control groups



The swimmers underwent three experimental trials. In the first trial, the swimmers and the control group, were tested in basal conditions (subjects were asked to abstain from strenuous physical activity for at least 24 h and to fast for 8 h before testing). Upon arrival to the laboratory, anthropometrical and body composition parameters: height, weight, body mass index (BMI), and body fat percentage (bioelectric impedance, OMRON BF300 analyser) were registered, and a blood sample (10 mL) was obtained from the antecubital vein. Subsequently, all subjects completed the State - Trait Anxiety Inventory (STAI) [28]. One week later, at approximately the same time of day (between 8:30 and 10:00 am), in the same resting and fasting conditions of the first trial, the swimmers participated in an official swimming competition. Before competition, and after 10 min. of rest in the swimming pool, a blood sample (10 mL) was obtained from swimmers, and they completed the STAI questionnaire (state version). This was followed by a standard warm-up (20 min. easy swimming). Immediately after the warm-up period, the swimmers competed in their respective 100m freestyle heats. Time to complete this distance was the mean of that registered by two official timekeepers. Immediately after each heat (1-2 min. post-exercise) the swimmers were subjected to another blood extraction (10 mL), and completing the STAI questionnaire (state version). The environment conditions registered in the competition were the following: 27.5° C for water temperature, 29.4° C for air temperature and 58% for relative air humidity (similar to first trial conditions).

Each blood sample was divided in two aliquots of 5 mL (EDTA K2 tubes with 50 µl of aprotinin: SIGMA A6279). One of the aliquots was destined to haematological analysis (Coulter JT3 analyser), and plasma was obtained from the remaining aliquot. Biochemical parameters measured in plasma samples were: PRL concentration (RIA, Radim-Ibérica. Intra-assay variation: 4%; inter-assay variation: 6%), total protein concentration (electrophotometry; Hitachi 737 autoanalyser, Boehringer-Mannheim) and lactate concentration (enzymatic determination and reflectance photometry method, Accusport®, Roche/Boehringer-Mannheim). Haemogram (haemoglobin concentration and haematocrit value) was performed to calculate the possible changes in plasma volume (PV) that could alter the results of the biochemical analysis [13]. Moreover, the plasma total protein concentration was determined in order to corroborate these changes in PV.

Data are expressed as mean \pm standard error of the mean (SEM). Non-parametric methods were used in statistical analysis. Friedman test was used to compare the experimental trials, and Mann-Whitney test was used to compare the variables between the experimental and the control group. Furthermore, linear



regression analysis was performed, and Pearson correlation coefficient was calculated to determine the relationship between variables. In all cases, statistical significance was established at an alpha level of 0.05.

Results

No significant differences were observed for plasma PRL and lactate concentration measured in basal conditions between the experimental and control groups. Similarly, no significant differences were observed in STAI scores registered in basal conditions between groups (Table 2).

Table 2

Plasma PRL and lactate concentrations and STAI scores registered in basal conditions

| | PRL (ng/mL) | Lactic acid (mmol/L) | STAI scores | |
|--------------|----------------|-------------------------|-------------|------------|
| | | | State | Trait |
| Experimental | 4.02±0.53 | 1.77±0.07 | 18.46±2.79 | 21.77±3.02 |
| Control | 4.36±1.09 | 1.72±0.07 | 1.72±0.07 | 22.10±3.63 |

Data represent means ± SEM.

A significant increase in plasma PRL concentration ($p \leq 0.05$) was observed in pre-competition compared with basal conditions (5.52 ± 0.53 vs 4.02 ± 0.53 ng/mL, respectively). However, plasma lactate concentration in pre-competition situation was similar to the concentration observed in basal conditions (1.89 ± 0.04 vs 1.77 ± 0.07 mmol/L, respectively) (Fig. 1 and Fig. 2). The STAI scores in pre-competition situation (24.5 ± 3.1) were significantly higher than those registered in basal conditions (Fig. 3), although no statistical relationship was established between PRL plasma concentrations and pre-competitive STAI scores ($r = -0.20$; $p = 0.95$).

Time to complete the 100 m freestyle was 61.47 ± 1.98 s. Interestingly, even though the competition was of such a brief duration, one finding of this study was the decrease in PV observed after the effort. This decrease was 11.42 ± 1.28 % compared to pre-competition values. In its computation, the haemoglobin concentrations as well as the haematocrit values pre- and post-competition were considered. The decrease in PV was supported by a statistically significant increase



of the plasma total protein concentration observed immediately after the competitive effort (Table 3). We used this individual percent change to correct each of the hormonal and biochemical determinations.

Table 3

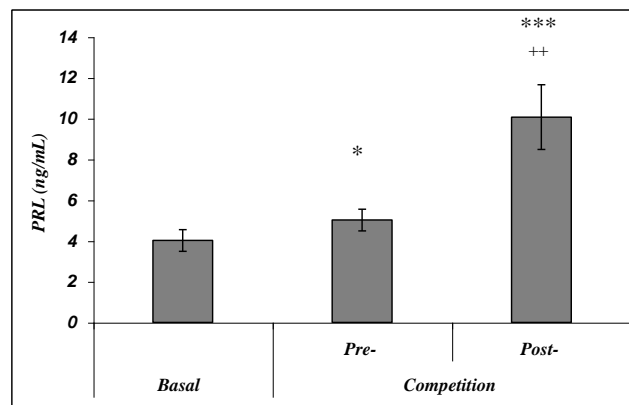
Biochemical parameters and changes in plasma volume

| | Official competition (100 m freestyle) | |
|-----------------------------|----------------------------------------|--------------------|
| | Pre – competition | Post – competition |
| Total plasma protein (g/dL) | 7.77±0.11 | 8.55±0.11*** |
| | 15.65±0.22 | 16.54±0.21 |
| Haemoglobin (g/dL) | | |
| Haematocrit (%) | 47.27±0.59 | 50.68±0.66 |
| PV change (%) | | - 11.42±1.28 |

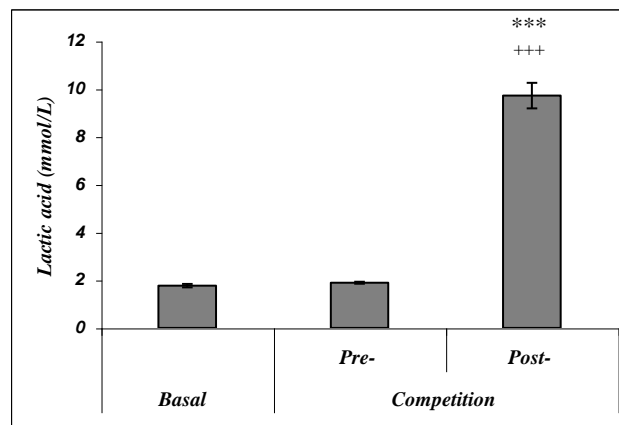
Data represent means ± SEM

The PRL response to the competitive effort produced a significant increase in the circulating levels of this hormone, reaching mean values of 10.07±1.59 ng/mL. These values were significantly higher than the ones found in basal conditions and those observed in the pre-competition situation (Fig. 1). Similar increases were observed in plasma lactate. Mean values of 9.72±0.53 mmol/L were registered post-competition. These values were also significantly higher than those found in basal conditions and observed before the swim (Fig. 2). However, regression and correlation analyses showed no relationship between PRL and plasma lactate concentrations in post-competition situation ($r=0.02$; $p=0.95$). On the other hand, the post-competition anxiety values (22.7±3.2), were slightly lower than those found before the beginning of the competitive swimming effort, and slightly higher than basal levels. No significant differences were observed with regard to these STAI scores (Fig. 3). A significant correlation ($p=0.04$) was found between PRL plasma concentrations and STAI scores after the swim, although the r value (0.57) showed a weak relationship.



**Fig. 1**

Swimmers' plasma PRL concentrations measured in all experimental trials. * $p \leq 0.05$; *** $p \leq 0.001$ compared with basal condition; ++ $p \leq 0.01$ compared with pre-competition trial

**Fig. 2**

Swimmers' plasma lactate concentrations measured in all experimental trials. *** $p \leq 0.001$ compared with basal condition; +++ $p \leq 0.001$ compared with pre-competition trial



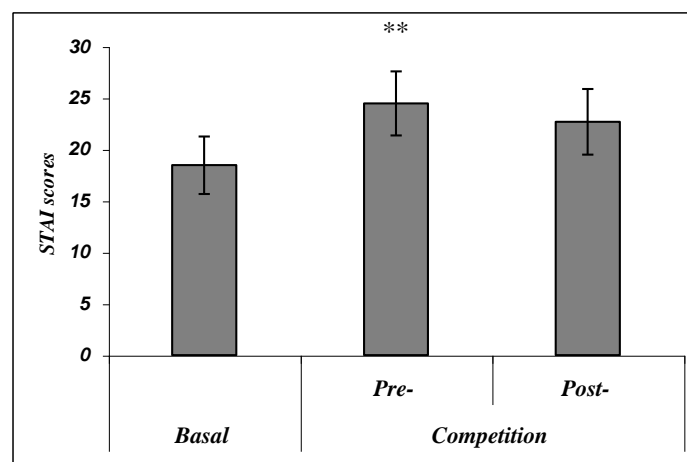


Fig. 3

Swimmers' state – anxiety levels according to the scores obtained in the STAI questionnaire in the three experimental situations. ** $p \leq 0.01$ compared with basal conditions

Discussion

From a psychosomatic perspective, there are few situations which can produce alterations such as those observed when an athlete has to undertake a maximal effort in competition. In the response to those alterations the endocrine system is promptly activated, and the hypothalamic-pituitary-adrenocortical (HPA) axis plays a key role in the response to stress, whether this stress has a somatic or a psychological origin (27).

PRL is released into the blood in response to different types of stress. For this reason, it's possible that the analyses of PRL plasma levels in basal situation can reflect an adaptation to stress induced by swimming training. PRL plasma concentrations obtained in basal conditions for both groups in this investigation are within the range proposed as normal reference values for adults (8) and are very similar to those registered in other studies (10).

With this results, and take into account that in a large number of investigations the PRL basal concentrations of trained and sedentary subjects are very similar [11,21,25] we concluded that this hormone levels are not useful to evaluate the adaptation to chronic stress induced by swimming training.

The mean plasma lactate values in basal conditions are higher than those reported in other studies with swimmers [3]. These values are, however, within the range described by the aforementioned authors (0.9-2.3 mmol/L).

Another important aspect to consider is the possible existence of psychological alterations (anxiety) in the subjects evaluated, since this could be the main trigger of the PRL release. Once the scores from the STAI questionnaire, with regard to trait-anxiety and state-anxiety were analysed, and after comparing them with the reference scale provided by Spielberger et al. [28] for the Spanish population, we conclude that the scores were close to the mean for this population. Although several studies have reported higher trait - anxiety levels in female swimmers than in amateur swimmers and sedentary sex and age matched groups [24], competitive swimming practice not seems to have an effect on swimmers evaluated in the present investigation.

An important PRL responses in swimming competition were registered. We found a significant increase (40%, $p \leq 0.05$) in PRL plasma concentration before taking part in the swimming competition compared to basal values. These results show how PRL responded to a situation where some degree of psychological stress (anxiety) was generated. An increase in the state-anxiety scores compared with the scores obtained in the basal condition ($p \leq 0.001$) verified this response. These increases in the anxiety levels are in accordance with those previously reported [20], although in these studies the pre-competition state-anxiety levels found in the swimmers were higher than the levels we have recorded. Nevertheless, there is no doubt about the relevance of the swimming competition analysed in our investigation since provoked an important rise in swimmer's anxiety levels. In this sense, the relevance of competition can be considered as a decisive factor in this type of investigations [23]. Our results are opposed to those reported by several authors whom not found PRL responses to pre-competition anxiety [25,29]. As expected, the plasma lactate values in the pre-competition situation (samples taken before warming-up) showed no significant differences from the basal values.

Immediately after finishing the swimming maximal effort (post-competition), significant increases in PRL plasma levels were registered. These values were up to 150% higher than basal concentrations ($p \leq 0.001$) and 82% higher than those found pre-competition ($p \leq 0.01$). This latter percent change could indicate the extent to which the physical (somatic) effort itself accounts for the PRL response. Although it have found PRL responses to progressive and maximal efforts [10,18,31], our findings, opposed to those obtained in previous studies [5], show an evident PRL response to high-intensity, short-term anaerobic exercise.



The plasma lactate concentration at the end of the competitive effort showed a marked increase compared to the basal values and to those obtained before the swimming bout ($p \leq 0.001$). These results were expected, since the 100 m freestyle swimming event is predominantly anaerobic the energy provision relies heavily on glycolytic metabolism, where large amounts of lactic acid are produced [6]. The observed lactate values were lower than those reported in other studies where the same sport and/or the same swimming distance was used [2]. These data refer to the peak lactate concentration, which requires several blood samples to be drawn during the post-exercise period to determine this peak precisely (some delay exists, since time is required for lactate to diffuse into blood from muscle cells). In the present investigation only one blood sample was obtained immediately after finishing the 100 m swimming event, so it is likely that we did not identify the peak blood concentration of this metabolite. The PRL response, as well as other hormones such as corticotropin and endorphins, can be stimulated by the lactate production [10]. In this regard, some authors have found a relationship between lactate and the plasma levels of these hormones after exercise [19,26]. A significant correlation between PRL and lactate plasma levels was not observed in the present study. Thus, our findings seem to dismiss a possible relationship between lactate accumulation and PRL response after short-term, maximal intensity exercise, conclusion that is opposite to those reported by other authors [9,21].

The state-anxiety scores obtained immediately after the swimming effort were, in general, lower than those registered pre-competition, although the differences were not statistically significant. Furthermore, there was no statistical difference between post-exercise and basal anxiety values, either, even though the post-exercise values were slightly higher. This decrease in anxiety observed after the exercise is in agreement with previous research, which showed lower anxiety levels after different types of exercise, even in those which were purely anaerobic [4].

Finally, a marked haemoconcentration was found after the maximal swimming effort. Indeed, the haematocrit and haemoglobin values experienced an increase which, expressed as percent change in PV, means a decrease exceeding 10%. Similarly, the significant increase observed in total plasma protein levels confirmed these changes in PV. These changes alone could have resulted in variations of post-exercise blood measures, although they were corrected. While, a priori, this kind of effort would not be capable of producing substantial modifications in PV (since it lasts only around one minute, and it is unlikely to be due to evaporative sweat), this exercise (taken into account the warm up) produced a considerable haemoconcentration. This result supports similar findings from another study showing a 16% decrease in PV after a 100 m maximal swim [16].



In conclusion, we can state that PRL basal levels do not change in response to competitive swimming training. Swimming competition (short-term maximal type of effort) induces a psychosomatic stress which stimulates the secretion of PRL. PRL response seems to be higher when stress has a somatic origin. Moreover, there was no direct relationship between PRL response and plasma lactate levels after high-intensity, short-term physical exercise. Future investigations are necessary to define the physiological role of PRL in this type of exercises.

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