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Research article

Salivary Biomarker Responses to Two Final Matches in Women's Professional Football

Javiera Maya [1], Pablo Marquez [1], Luis Peñailillo [1], Ariel Contreras-Ferrat [1], Louise Deldicque [2] and Hermann Zbinden-Foncea[1]✉
[1] Exercise Science Laboratory, School of Kinesiology, Faculty of Medicine, Universidad Finis Terrae, Santiago, Chile
[2] Institute of Neuroscience, Université catholique de Louvain, Louvain La Neuve, Belgium

Abstract
The aim of this study was to examine the link between salivary concentrations of cortisol, testosterone, immunoglobulin A (IgA) and the rate of perceived exertion (RPE) as a measure of internal load after two final matches played 3 days apart by professional women football players. Saliva samples were taken before and after the two matches (M1, M2). RPE was used to monitor the exercise intensity after each match. Testosterone concentrations increased after each match (M1: +42%, $p = 0.002$; M2: +50%, $p < 0.001$) while cortisol increased only after M1 (+116%, $p < 0.001$). The testosterone-to-cortisol ratio decreased only after M1 (-32.4%, $p < 0.001$). IgA concentration did not change after any match. Testosterone concentrations were correlated with IgA concentrations after each match (M1: $R = 0.59$, $p = 0.008$; M2: $R=0.51$, $p = 0.02$). RPE was correlated with cortisol concentrations after M1 ($R = 0.57$; $p = 0.01$), but not after M2 ($R = 0.38$; $p = 0.07$). All these results suggest that salivary cortisol and testosterone concentrations increase especially after the first match of a final, without affecting IgA levels. We speculate that increased testosterone concentration in women after football matches may play a protecting role against immune suppression usually observed after intense exercise.

Key words: Soccer, hormones, saliva, T/C ratio, RPE, stress, immune response.

Introduction

Several studies have reported a relationship between physical and psychological stress with changes in hormonal concentrations such as cortisol and testosterone (Doan et al., 2007; Greig et al., 2006) and immune function (Moreira et al., 2013). However, the majority of these studies have been performed in men, thus the evidence of the hormonal response after exercise in women is scarce (Moreira et al., 2009; Oliveira et al., 2009; Stolen et al., 2005).

Men studies consistently reported that testosterone and cortisol are highly responsive hormones to exercise (Banfi and Dolci, 2006; Gaviglio et al., 2014). Indeed, exercise-induced stress is associated with the stimulation of the hypothalamic corticotropin-releasing hormone, release of pituitary adrenocorticotropic hormone and finally production of the adrenal glucocorticoids, such as cortisol (Edwards and Casto, 2013; Moreira et al., 2009). In men, cortisol concentrations have been shown to increase after both individual and team events (Doan et al., 2007; Filaire et al., 2009; Filaire et al., 2001; Gonzalez-Bono et al., 1999). Many studies in men used a simulated competition paradigm, but cortisol regulation seems to be dependent on real situation instead, probably as a response to psychological stress (Moreira et al., 2009). To the best of our knowledge, only one study has compared the levels of salivary cortisol in women after a training match and a competitive football match (Haneishi et al., 2007). These authors showed that salivary cortisol post-competitive match was increased, but no significant changes were observed after the training match, suggesting that psychological stress during the competition plays a role in the increase in cortisol concentration (Haneishi et al., 2007). Furthermore, Moreira et al. (2012) reported a significant correlation ($r = 0.75$) between the rate of perceived exertion (RPE) scores, as a measure of internal load, and the salivary cortisol responses of professional male basketball players during both simulated and official matches (Moreira et al., 2012). RPE has been shown to be a valid marker of exercise intensity and internal load under a variety of conditions and situations (Costa et al., 2013). RPE has been compared with heart rate and oxygen consumption and proved to be an efficient tool for monitoring intensity of exercise as a physiological load (Scott et al., 2013). However, the association between RPE and the hormonal responses after an official football match in women has not yet been investigated.

Testosterone is an anabolic androgenic steroid that is produced by the Leydig cells in the testis, ovaries and zona reticularis of the adrenal cortex (Vingren et al., 2010). In some, but not all studies, testosterone has been suggested to play a crucial role in muscle growth and strength in both men and women in response to resistance training (Vingren et al., 2010). Interestingly, whether testosterone concentrations increase in women after other sport activities seems quite controversial. Obviously, the potential increase in testosterone after exercise should be explained by different mechanisms in a gender-dependent manner. In men, the Leydig cells are mainly responsible for this increase (Wheeler et al., 1994), whereas in women exercise-induced increases in testosterone could be an indirect consequence of increased cortisol and adrenocorticotropin (ACTH) levels as the latter stimulates adrenal production and release of testosterone (Vingren et al., 2010). Therefore, it is possible that testosterone concentrations may also increase post-exercise even in female athletes, however it has not been tested so far whether this is the case in response to a competitive football match.

Furthermore, the testosterone to cortisol ratio (T/C

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ratio) is usually used to estimate the anabolism/catabolism balance of skeletal muscle in athletes (Crewther et al., 2006). This ratio gives a simplistic insight into a complex physiological system that, upon an increase in stress, experiences a reduction in testosterone and an increased cortisol secretion (Banfi and Dolci, 2006), decreasing the T/C ratio. For instance, a decrease in T/C ratio indicates a potential increase in catabolic metabolism in skeletal muscle, which raises an important issue for elite footballers, who rely on muscular strength to perform high-intensity, explosive and powerful actions. However, this hormonal response, i.e. the changes of the T/C ratio, during competitive matches in female footballers is still unknown.

In addition to increased cortisol and decreased testosterone concentrations, intense exercise can also affect immunoglobulin A (IgA) secretion (Putlur et al., 2004). Salivary IgA concentrations have been shown to decrease after exercise in men (Gleeson et al., 1999; McDowell et al., 1991) and women (Martins et al., 2009; Schouten et al., 1988). IgA is considered as the first barrier against microorganisms that cause upper respiratory tract infections (Corthesy, 2009). Exercise-induced decreases in IgA concentrations increases the risk of contracting infections of the upper respiratory tract due to immune function suppression (Moreira et al., 2013). Sports scientists have used salivary IgA to study the relationship between the immune function, respiratory tract infections and exercise intensity, but conflicting results have been obtained (Moreira et al., 2009; Neves Sda et al., 2009; Sloan et al., 2013). Consequently, the usefulness of determining salivary IgA after exercise remains under debate due to the lack of robust data, especially in women.

Therefore, the aims of this study were to determine the changes in cortisol, testosterone and IgA concentrations and to explore the relationship between these changes and the perception of exertion as a measure of internal load after a tournament final consisting in away and home matches in professional female football players. We hypothesized that both salivary cortisol and testosterone concentrations would increase, while salivary IgA concentration would decrease after each match and that those changes would be related to perceived exertion after the matches.

Methods

Study design

Saliva and RPE were collected before and after the final of a professional women´s football tournament consisting in two matches (home and away) based on cumulative goal differential. During the first match, team A (playing away) defeated team B (playing home) by 2-0. In the second match, team A (playing home) won 4-0 against team B. Data were collected from both teams. Each match consisted of two 45-min periods with a 15-min half-time break. The time between match 1 and 2 (M1 & M2) was three days during which the players only recovered and exercised at low-moderate intensity. M1 was played at 5:30 pm and M2 was played at 12:30 pm.

Participants

Initially, 22 female players of both teams were included in this study, of which six were excluded of the analyses because they did not play both matches. Thus, the samples of 16 professional female football players (mean ± SD; age: 22.5 ± 2.1 years, height: 1.63 ± 0.07 m, weight: 59.5 ± 6.3 kg, and body mass index: 22.6 ± 2.2 $kg \cdot m^{-2}$) were finally analysed. Both teams belonged to the women´s first division of the Chilean Professional Football League, which is the highest division in Chile. In our study, neither the menstrual cycle phase, nor the use of oral contraceptives was monitored or controlled, reflecting the real situation of a competition. At the time of the experiment, all players regularly trained 120-150 min per session, one session per day, 5 days a week, and played on average one competitive match per week. The study was approved by the ethics committee of the Universidad Finis Terrae and conformed to the principles outlined in the Declaration of Helsinki. All players gave their written informed consent to participate.

Saliva collection

Saliva samples were collected 30 min before the match (Pre-) and 5-10 min after (Post-) the player was substituted or the match was ended. Initially, players were required to rinse out their mouth with distilled water in order to prevent foods with a high acid or sugar content from compromising the samples. Unstimulated whole saliva (3 ml) was collected into a 10-ml plastic sterile bag and placed into an ice container. The procedure took approximately 5 min in total. Saliva flow rate was not determined. Samples were later centrifuged at 1,500 g for 15 min and the supernatant was stored frozen in microtubes at -20º C until samples were assayed (Moreira et al., 2009).

Saliva analysis

Saliva samples were taken due to their easy collection, low invasivity and easy identification of the free active compounds in the sample, such as cortisol, testosterone and IgA, which are correlated with their free blood plasma form (Gatti and De Palo, 2011; Lewis, 2006). Enzyme-linked immunosorbent assays (ELISA) were used to analyse cortisol, testosterone and IgA concentrations according to the manufacturer's protocol (Salimetric, USA). All the analyses were performed in duplicate. Furthermore, testosterone to cortisol concentrations (T/C) ratio was also calculated. The coefficient of variation of the intra-assays in the present study was 3.10%, 4.09% and 3.67% for cortisol, testosterone and IgA, respectively.

Rate of perceived exertion

Since no devices were allowed to measure physiological parameters such as heart rate, distance covered, average speed during official matches, we used the changes in RPE after each match to assess internal load of the players. Participants were asked to rate their RPE after the match according to the Borg´s category ratio (CR-10) scale (Castagna et al., 2007). A numbered scale ranging from 1 to 10 was shown to the players, in which each

number was assigned to a fixed exertion level, ranging from extremely easy (1) to extremely hard (10).

Statistical analysis

Data are presented as means and standard deviations (mean ± SD). To determine the distribution of the data a Shapiro-Wilk test was performed prior statistical model selection. Shapiro-Wilk test found that cortisol, testosterone and IgA variables were normally distributed. To compare the rates of perceived exertion between matches a paired t-test was used. To compare the concentration of cortisol, testosterone and IgA before and after each match, a two-way analysis of variance (ANOVA) with repeated measured was used. If a significant match, time or interaction effect was found a Fisher´s LSD post hoc test was used for pairwise comparisons. Furthermore, Cohen´s d effect size (ES_d) and partial-eta squared effect sizes ($ES_{\eta P}^2$) were used for t-test and repeated measures ANOVA analyses, respectively. Threshold values for assessing magnitudes of ES were 0.20, 0.60, 1.2, and 2.0 for small, moderate, large, and very large, respectively (Hopkins, Marshall, Batterham, & Hanin, 2009). In addition, we have provided the 95% confidence intervals (CI) as suggested by Hopkins et al. (2009) as shown in Table 1. A Pearson product-moment correlation coefficient was used to assess the relationship between the post-match salivary concentrations and RPE values after each match. All statistical analyses were performed with PASW Statistics 19 software for Mac (SPSS Inc., IBM Company, USA). The significance level was set at $p < 0.05$.

Results

RPE

Post-match RPE was similar (p = 0.58, CI = -0.894 – -0.519, ES_d = 0.05) between M1 and M2 (5.63 ± 2.13 and 5.81 ± 1.42, respectively).

Changes in salivary cortisol, testosterone and immunoglobulin A concentrations

There was not differences in the concentrations of cortisol, testosterone and IgA between teams A and B, hence data of both teams were pooled together (n=16). According to the two-way ANOVA analyses, cortisol concentrations showed a significant interaction (match x time) effect (F(1) = 8.466, p = 0.01, $ES_{\eta P}^2$ = 0.36), which was not the case for testosterone (F(1) = 0.16, p =0.9, $ES_{\eta P}^2$ = 0.001) and IgA (F(1) = 0.095, p = 0.8, $ES_{\eta P}^2$ = 0.006) concentrations (Table 1). T/C ratio also showed a significant interaction effect (F(1) = 21.09, p = 0.0001, $ES_{\eta P}^2$ = 0.58). Cortisol (F(1) = 34.006, p = 0.0001, $ES_{\eta P}^2$ = 0.69), testosterone (F(1) = 28.128, p = 0.0001, $ES_{\eta P}^2$ = 0.65) and T/C ratio (F(1) = 5.86, p = 0.03, $ES_{\eta P}^2$ = 0.28) showed a significant time effect. IgA concentration did not change over time (F(1) = 0.033, p = 0.86, $ES_{\eta P}^2$ = 0.002), neither in M1 nor M2.

Pairwise comparisons are shown in details in Table 1. It can be seen that cortisol concentrations significantly increased by 53.9% after M1, but did not increase after M2, although a trend to increase was present (25.8%). Cortisol concentrations tended to be higher before M2 than before M1 without reaching the statistical threshold. Testosterone levels increased significantly from pre- to post-match in both M1 and M2 (29.8% and 32.8%, respectively). No difference in testosterone concentration was observed between M1 and M2 both before and after match. No difference in IgA concentrations were observed in any match-play (Table 1). T/C ratio significantly decreased by 31.9% after M1, but did not change after M2.

Table 1. Pre- and Post-match mean (±SD) values for salivary concentrations together with 95% confidence interval and effect size.

		Match 1	Match 2
Cortisol (nmol·L^{-1})	Pre-	10.18 (1.54)	12.24 (4.49)
	Post-	22.07 (7.03)	16.49 (7.70)
	% Diff	+53.9%	+25.8%
	p-value	<.001	.058
	95% CI	8.22 – 15.56	.16 – 8.69
	Effect size	0.75	.32
Testosterone (nmol·L^{-1})	Pre-	.27 (.07)	.25 (.36)
	Post-	.39 (.14)	.36 (.16)
	% Diff	+29.8%	+32.8%
	p-value	.002	<.001
	95% CI	.05 – .17	.06 – .17
	Effect size	.46	.42
IgA (nmol·L^{-1})	Pre-	347.6 (172.6)	334.6 (101.0
	Post-	335.0 (154.6)	330.9 (162.9)
	% Diff	-3.7%	+1.3%
	p-value	.74	.91
	95% CI	-35.53 – 25.80	-69.43 – 77.74
	Effect size	.04	.01
T/C ratio	Pre-	.22 (.08)	.17 (.07)
	Post-	.15 (.05)	.19 (.08)
	% Diff	-31.9%	+10.5%
	p-value	<.001	.18
	95% CI	-.09 – -.04	-.01 – .05
	Effect size	.53	.13

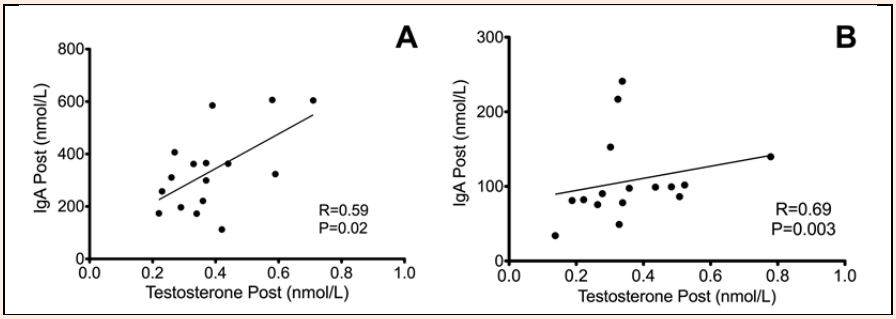

Figure 1. **Correlation between testosterone and IgA levels after (A) match 1 (M1) and (B) match 2 (M2).**

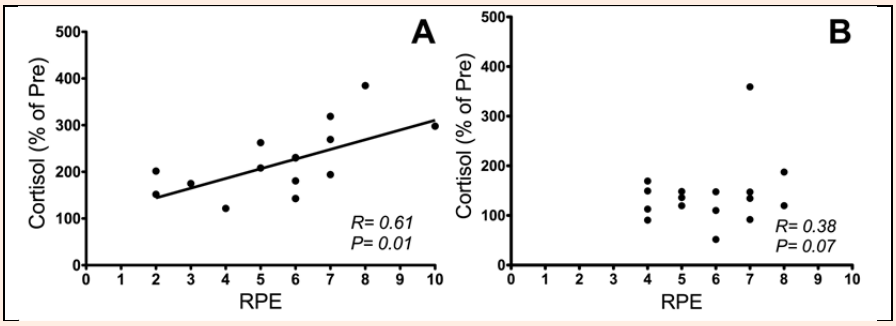

Figure 2. **Correlation between cortisol and the rate of perceived exertion (RPE) after (A) match 1 (M1) and (B) match 2 (M2).**

Correlation between RPE and salivary hormone concentrations

A positive correlation between testosterone and IgA concentrations after M1 (r = 0.59; p = 0.02, Figure 1A) and M2 (r = 0.69; p = 0.003, Figure 1B) was found. A positive correlation was also found between RPE values after each match and the changes in cortisol concentrations (% of Pre) after M1 (r = 0.57; p = 0.008, Figure 2A), but only a tendency to a positive correlation was observed after M2 (r = 0.38; p = 0.07, Figure 2B).

Discussion

The main findings of this study were that two final women football matches (M1 & M2) resulted in: (1) significantly greater increase in salivary cortisol concentrations after the first match of the final, while testosterone increased similarly after both matches; (2) a decreased T/C ratio after the first match, which may reflects a greater psychological stress of the first match of a tournament´s final and; (3) a maintenance of salivary IgA concentrations after both matches. These results are partially in line with our hypotheses since salivary cortisol increased only after the first match and testosterone concentrations increased after both matches, concomitantly T/C ratio decreased after the first match only. However, salivary IgA concentrations did not change after any match, while a decrease was hypothesised. Furthermore, only salivary cortisol concentrations after the first match of the final were correlated with RPE, indicating that hormonal secretion is poorly related with the perceived internal load of matches.

It is important to note that this study was conducted in two competitive official matches that would determine the champion of the tournament. Haneishi et al. (2007) demonstrated that real competition induces greater hormonal responses in female athletes when compared to those obtained from laboratory exercises. Moreira et al. (2013) found cortisol concentrations to be affected to a greater extent during a formal than a friendly match in men. In the present study, salivary cortisol concentrations were doubled after the first match, but only a tendency to increase after M2 was observed. This different response could be due to the importance of the first match of a final, but also along with various other stress factors such as the situation of the match and the interaction with other players experienced during M1 (Greig et al., 2006). In line with this, it seems that the psychological stress contributes importantly to the raised cortisol concentrations in athletes (Doan et al., 2007). Thus, the different magnitude of increase in cortisol concentration observed after M1 and M2 can be explained by the relative importance of M1 and difference in the score obtained after the first match by the winning team (2-0). Since the score in M1 was large enough (2-0), we speculate that less stress was induced during the second leg of the final (M2) in both teams compared to M1. It is possible that the winning team tactically controlled the match, which induced a different response in players than in M1.

Testosterone concentrations increased to a similar extent after each match, i.e. ~50%. Given that in women the adrenal gland is the principal source of testosterone, and that cortisol and testosterone have a common origin, the response of both hormones should have followed the same trend (Stanton, 2011). A recent study in women suggests that salival cortisol and testosterone concentrations rise in parallel during competition and that increases in concentration of one hormone are significantly related

to the increases in the other (Edwards and Casto, 2013). However, this was not the case in this study, since cortisol only showed a trend to increase of 25.8% after M2. It was previously postulated that the main sources of increased testosterone after a match in women is a higher secretion from the adrenal glands and the conversion of androgenic precursors, the secretion of which is stimulated by ACTH that also triggers the secretion of cortisol (Edwards and Casto, 2013). It was therefore somewhat surprising that testosterone and cortisol concentrations responded differently after M2. However, it has been suggested that ovaries could also contribute to competition-related increases in testosterone (Edwards and Casto, 2013) and thereby enhance adrenal secretion, independently of cortisol and ACTH. In summary, some common mechanisms regulate the secretion of cortisol and testosterone, but also independent mechanisms could contribute to the changes observed and can explain the small different regulation between cortisol and testosterone that we observed after M2. Interestingly, we did not find differences in the increases in testosterone between matches nor between the winning and losing teams, whereas it has previously been shown that post-match testosterone concentrations were higher in the winning than in the loosing team (Neave and Wolfson, 2003). Furthermore, we did not find any difference between the winning and losing teams for cortisol and IgA. Hence, the data of both teams, winner and loser, were pooled together.

The T/C ratio is often calculated to monitor the anabolic/catabolic status of an athlete (Crewther et al., 2006). A decrease in T/C ratio was observed after M1 although no change was observed after M2 when compared with the respective pre-match values. This decrease after M1 resulted from a greater rise in cortisol than in testosterone concentrations. This could be interpreted as the catabolic status being higher than the anabolic status post- compared to pre-M1. No change was observed between pre- and post-match values during M2, but notably pre-values were lower and post-values higher in M2 compared with M1. While pre- and post-match testosterone concentrations were rather similar, the cortisol concentrations differed between both matches and explained the changes in the T/C ratio between pre- and post-match conditions. Therefore, it seems that cortisol changes mostly dictated the changes in the T/C ratio in the present study and that psychological factors could have influenced this ratio.

From our results, we speculate that the increases in testosterone concentrations might help to limit the IgA concentration reduction typically reported after intensive physical exercise. Androgens, such as testosterone, have been related to a protective effect over immunosuppresion in animal and in vitro models (Grossman, 1985). In addition, males of some species tend to be less prone to infections and have a reduced incidence of autoimmune diseases than women (Klein, 2000). However, in healthy humans, studies have provided conflicting evidence regarding this protective effect of testosterone (Vingren et al., 2010). In the present study, we found a direct and positive correlation between salivary testosterone and IgA, both after the first and second match. Whether testosterone itself regulates the changes in IgA or is it the transformation of testosterone into estrogen that matters is difficult to know from this study. Indeed, salivary IgA concentrations have been found to be correlated to estradiol concentrations in women at rest (Van Anders, 2010). Thus, it can be speculated that in our study, the correlation between testosterone and IgA concentrations is only an indirect effect of testosterone via transformation into estradiol. Although it is well known that IgA concentrations decrease after intensive exercise (Owen et al., 2014), this was not the case in the present study, thus, we hypothesise that testosterone could exert some kind of immunoprotection post-exercise in women.

Perceived exertion after the match was similar between M1 and M2. From all variables measured, RPE only correlated with cortisol concentrations after M1 and only tended to correlate after M2. As mentioned earlier in the discussion, changes in cortisol concentrations after a women's football match seem not to be the sole result of physical stress, but possibly of psychological stress as well. However, RPE may reflect internal load only. A limitation of the present study is that no markers of external load or direct physiological measures were taken because equipment to assess physiological parameters were not allowed in official football matches at that time. Therefore, it is difficult to assess if internal or external load influences the regulation of cortisol, testosterone and IgA in the present study.

Undoubtly the real competition situation of the present study is an added value, but it is also accompanied by some difficulties. Usually, studies in women control the specific moment of the menstrual cycle where exercise is performed, but in the present study it was impossible to control the menstrual cycle phase of the players. Deliberately, we decided not to standardise the cycle phase to reflect the real situation in which women compete. This choice limits somewhat the physiological interpretation of the results, but extends the validity of the results on the field.

Conclusion

In conclusion, the results of the present study indicate that a women's football match increases salivary cortisol and testosterone concentrations especially after the first match of a final, without affecting IgA concentrations. We speculate that increased testosterone concentrations after football matches may play a role in protecting against the immune suppression usually observed after exercise, but this requires further investigation.

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Key points

- In our sample space, IgA concentrations did not change for teams even, before and after separated match. Suggesting that salivary IgA determinations after physical activities remain under debate.
- Testosterone concentrations were the only one hormone showing a consequent increase in both matches after physical activity carrying.
- The T/C ratio decrease only after M1 according with a higher cortisol level reach after M1 get-together, suggesting a differential impact over anxiety-associated team performance. So M2 play gives a more stable psychological state.

AUTHOR BIOGRAPHY

Javiera MAYA
Employment
Graduate student, Universidad Finis Terrae
Degree
MSc
Research interest
Clinical exercise physiology
E-mail: javimaya@gmail.com

Pablo MARQUEZ
Employment
Graduate student, Universidad Finis Terrae
Degree
MSc
Research interest
Clinical exercise physiology
E-mail: pmarquezn@gmail.com

Luis PEÑAILILLO
Employment
Professor (Assistant) of exercise and sport science. Universidad Finis Terrae
Degree
PhD
Research interest
Exercise physiology and resistance/strength training
E-mail: lpenailillo@uft.cl

Ariel CONTRERAS-FERRAT
Employment
Professor (Assistant) of physiology. Universidad Finis Terrae
Degree
PhD
Research interest
Skeletal muscle physiology
E-mail: arielcontrerasf@gmail.com

Louise DELDICQUE
Employment
Professor in exercise physiology. Université catholique de Louvain.
Degree
PhD
Research interest
Endurance physiology and nutrition
E-mail: louise.deldicque@uclouvain.be

Hermann ZBINDEN-FONCEA
Employment
Professor (Associate) of exercise physiology and sport science. Universidad Finis Terrae
Degree
PhD
Research interest
Exercise physiology, chronic diseases and exercise
E-mail: hzbinden@uft.cl

✉ **Hermann Zbinden-Foncea, PhD**
School of Kinesiology, Universidad Finis Terrae, 1509 Pedro de Valdivia Avenue, Providencia, Santiago, Chile