Acupuncture and Responses of Immunologic and Endocrine Markers during Competition

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ABSTRACT

AKIMOTO, T., C. NAKAHORI, K. AIZAWA, F. KIMURA, T. FUKUBAYASHI, and I. KONO. Acupuncture and Responses of Immunologic and Endocrine Markers during Competition. *Med. Sci. Sports Exerc.*, Vol. 35, No. 8, pp. 1296–1302, 2003. **Introduction:** Acupuncture is used to modulate the physical well-being of athletes in Asian countries. However, there is little information on the immediate effects of acupuncture treatment on physiological or psychological responses to exercise. **Purpose:** The purpose of this study was to examine the effect of acupuncture treatment on the physical well-being of elite female soccer players during a competition period. **Methods:** Subjects were divided into two groups: those who received acupuncture treatment (18.1 ± 2.3 yr [±SD], $N = 9$) and a control group (17.7 ± 2.8 yr, $N = 12$). In the treatment group, acupuncture stimulus was applied at LI 4 (Goukoku), ST 36 (Ashi-sanri) for 20 min, and ST 6 (Kyosya), LU 6 (Ko-sai) points for 15 min 4 h after the game every night during the competition period. The measured parameters included salivary secretory immunoglobulin A (SIgA) level, cortisol level in saliva, subjective rating of physical well-being, and profile of mood states (POMS). **Results:** The following were the main results: 1) Exercise-induced decrease of salivary SIgA and increase of salivary cortisol were inhibited by acupuncture. 2) Acupuncture improved subjective rating of muscle tension and fatigue. 3) The POMS score was modulated by acupuncture. **Conclusion:** These results support the effectiveness of acupuncture for physical and mental well-being of athletes. **Key Words:** SALIVA, IgA, CORTISOL, POMS

Professional and amateur athletes sometimes receive acupuncture for treatment of a variety of ailments. Especially in Asian countries, acupuncture has been used to modulate the physical well-being of various athletes (18). The intention is to achieve a higher level of competitive performance (24). Acupuncture is defined as the penetration of the skin with stainless steel needles, which stimulate the tissue manually, electrically, or by heat. Experiments show that acupuncture treatment produces various effects including alleviation of muscle tension, improvement of local blood flow, increase of pain threshold, and modulation of autonomic nervous system (3,13). In fact, acupuncture has been used for treatment of injury, reduction of fatigue, and management of physical condition in athletic fields (10,18). However, there are a few scientific studies on the physiological effects of acupuncture treatment on the physical well-being of athletes (24). The physical well-being of athletes is considered to be the critical physical and mental condition for optimal athletic performance. Physical well-being is regulated by three regulatory systems in the human body, i.e., nervous, endocrine, and immune systems. Several parameters related to these systems are influenced by intense exercise, prolonged training, and overtraining (7).

Several studies have shown that a single session of intense exercise and prolonged heavy training negatively influences the immune system function (23). Recent studies have demonstrated that frequent upper respiratory tract infections (URTI) in elite athletes result in suppression of salivary secretory immunoglobulin A (SIgA) levels (8,16). SIgA is the predominant immunoglobulin in secretions of the mucosal immune system and is found in the saliva, intestinal secretions, bronchoalveolar lavage fluid, urine, tears and other mucosal fluids. SIgA neutralizes various toxins and viruses, and inhibits the attachment and replication of pathogenic microorganisms, and therefore prevents colonization of these pathogens (16).

The endocrine system responds to physical and mental stress including intense exercise, continuous intense training, and competitive stress. Cortisol is a stress marker secreted from the adrenal cortex by physical and mental stress (20). The level of secretion is affected by the type, intensity, and duration of exercise.

It has been shown that the immune and endocrine systems of the body can interact with each other. The immune and endocrine systems communicate bidirectionally via shared messenger molecules, variously called cytokines and hormones (25). It is therefore important to monitor immunologic and endocrine markers for objective assessment of the physical well-being of athletes. Because measurement of

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TABLE 1. Subjects' characteristics.

	Treatment group ($N = 9$)	Control group ($N = 12$)
Age (yr)	17.7 ± 1.4	18.1 ± 1.2
Height (m)	1.593 ± 0.018	1.581 ± 0.019
Weight (kg)	54.0 ± 2.6	52.4 ± 2.9
Body fat (%)	21.2 ± 1.7	20.9 ± 1.8
BMI	20.4 ± 1.6	20.2 ± 1.1

* BMI, body mass index; data expressed as mean ± SE.

TABLE 2. Total playing time in each group.

	Treatment Group ($N = 9$)	Control Group ($N = 12$)
Day 1	96.6 ± 11.0	77.3 ± 9.5
Day 2	113.8 ± 19.0*	74.1 ± 7.6
Day 3	109.2 ± 11.0	97.3 ± 9.9
Total	105.6 ± 13.6	82.9 ± 9.0

Data expressed as mean ± SE.
* $P < 0.05$ (between groups).

certain salivary components by using noninvasive and stress-free method is now possible, it should be easy to determine the physiological response to exercise, training and competition (4).

The purpose of this study was to determine the effect of acupuncture treatment on physical and mental condition of elite female soccer players by measuring salivary SIgA and cortisol levels during a period of sports competition. We hypothesize that acupuncture treatment would inhibit a decrease of physical well-being induced by physical exercise. For this purpose, we measured the changes in salivary SIgA and cortisol as objective markers of immunologic and endocrine system function.

MATERIALS AND METHODS

Subjects. Twenty-one elite female soccer players who took part in an international soccer competition (July 2001) were subjects of the present study. They were randomly divided into two groups (Table 1). Nine players (18.1 ± 2.3 yr old) in the treatment group received acupuncture treatment. Twelve players (17.7 ± 2.8 yr old) who were not treated acted as the control group. There were no differences in age, body weight, and height and body mass index. All subjects had normal menstrual cycles and did not use oral contraceptives.

Figure 1 shows the experimental design. The subjects were tested over a 3-d period that involved six games. The first game started at 8:30 a.m. and the second started at 2 p.m. on day 1. The third and fourth games (day 2) and fifth and sixth games (day 3) started at same time as day 1. Each game consisted of two 35-min halves, and the playing time of each subject is given in Table 2. The playing time was calculated using a game logbook. After the second game of the day, subjects took a bath, dinner, and rest in their room. Acupuncture treatment was administrated by turns from 8 p.m. to 10 p.m., 1 d before and during the competition. It consisted of placement of disposable 2-gauge stainless needles (40 mm long, SEIRIN, Shizuoka, Japan) at LI 4 (Goukoku) and ST 36 (Ashi-sanri) with 2-Hz electrostimulation (Ohm pulser, Zen Iryoki, Fukuoka, Japan) for 20 min. At the same time, ST 6 (Kyosya), LU 6 (Ko-sai) were also stimulated bilaterally with aurical needles (SEIRIN Jr, SEIRIN) for 15 min during the electroacupuncture treatment (Fig. 2). The general effects of each point include coordination of whole body (LI 4, ST 36), stimulation of salivary gland secretion (LU 6), and treatment and prevention of common cold (ST 6) (Table 3). Acupuncture was performed by a licensed acupuncturist with 10-yr experience in acupuncture treatment.

The study protocol was in accordance with the policy statement of the institutional review board of University of Tsukuba. All subjects had passed a complete medical examination within the past year and received written permission from a specialist sports physician to participate in the study. All subjects submitted informed written consent to the institutional review board. Subjects did not use medications known to affect immune function before and during the study. None of the subjects had allergies or acute infection at the time of the study.

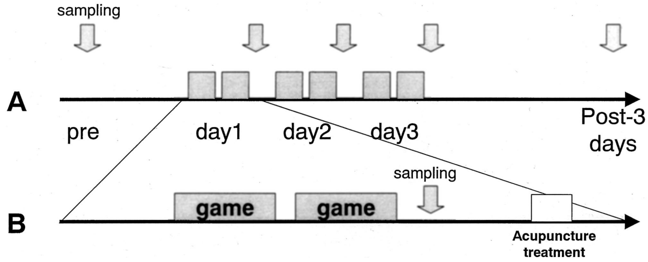

FIGURE 1—The experimental design of this study. A. The experimental schedule during this study. B. The schedule of an experimental day. Saliva samples were collected at 6 p.m. (*arrows*), 1 wk before (pre), during 4 d (d 1–3) of competition, and 3 d after the competitive period (post-3 d). Subjective rating of physical condition and POMS were filled out after the salivary samples. Acupuncture treatment was administrated from 8 p.m. to 10 p.m., 1 d before and during the competition (3 d).

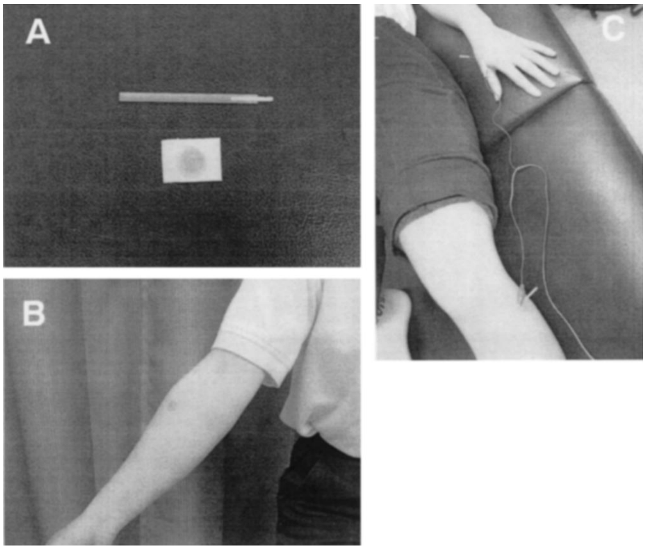

FIGURE 2—Needles for acupuncture. A. Two types of needles for acupuncture were used in this study. B. LU 6 (Ko-sai) on both side were also stimulated with aurical needles (SEIRIN Jr, SEIRIN). C: Electroacupuncture at ST 36 (Ashi-sanri) with 2-Hz electrostimulation (Ohm pulser, Zen Iryoki).

TABLE 3. Acupuncture points used in this study.

Acupuncture Point	General Effects	Location
LI 4 (Goukoku)	Coordination of whole body	On the dorsum of the hand, between the first and second metacarpal bones, at the midpoint of the second metacarpal bone, and close to its radial border
ST 36 (Ashi-sanri)	Coordination of whole body	One finger-breadth from the anterior crest of the tibia in m. tibialis anterior
ST 6 (Kyosya)	Treatment and prevention of common cold	Approximately 1 fingerbreadth anterior and superior to at the angle of the jaw at the prominence of the masseter muscle
LU 6 (Ko-sai)	Stimulation of salivary gland secretion	On the flexor aspect of the forearm

Saliva samples. Saliva samples were collected at 6 p.m., 1 wk before (pre), during 4 d (days 1–3) of competition, and 3 d after the competition period (post-3 d). Food intake was prohibited 5 h before salivary collection, but the subjects were free to drink water. Saliva samples were collected as described previously (1). Briefly, the mouth was first rinsed thoroughly with distilled water followed by stimulation of saliva secretion with chewing a sterile cotton swab (Salivette, Sersted, Germany) at a frequency of 120·120 s^{-1}. Saliva was separated from the cotton by centrifugation at 3000 rpm. After measurement of the sample volume, saliva samples were frozen at −80°C and stored until analysis.

SIgA levels. Salivary SIgA concentrations were measured by enzyme-linked immunosorbent assay as described previously (1). Briefly, a 96-well microtiter plate (Immulon II, Dynex Technologies, Chantilly, VA) was coated with rabbit antihuman secretory component IgG fraction (MBL, Nagoya, Japan) overnight at 4°C. After adding 250-μL phosphate-buffered saline (PBS) containing 1% bovine serum albumin (BSA; Sigma, St. Louis, MO), wells were blocked for 2 h. Saliva samples were thawed, centrifuged at 10,000 rpm for 5 min and diluted (1/20) with PBS containing 1% BSA, and 100 μL of each were added and incubated for 1 h. Using purified human SIgA (Organon Teknika, Durham, NC), known concentrations of SIgA were also plated to establish standard values. After washing the plate with PBS-Tween, goat Fab' anti-IgA conjugated with HRP (MBL) was added to the plate and incubated for 1 h. After washing, substrate solution was added and the color intensity produced after 15 min was measured by a microplate reader (Bio-Rad Laboratories, Hercules, CA) at 490 nm. All samples were assayed in duplicate and the average of absorbance values was used as the representative value. Calibration curves were constructed using standard SIgA concentrations and the concentration of SIgA in the samples was determined from these curves. The intra-assay variability were $<$ 5% on duplicate samples. To avoid interassay variability, all samples from each subject were assayed on the same plate. The interassay coefficient of variation of the method based on analysis of 82 duplicate samples was 6.2%. The concentration of total protein in the saliva was measured by using the method of Bradford (Bio-Rad Laboratories).

Cortisol levels. Salivary levels of cortisol were analyzed by radioimmunoassay using a commercially available kit (Diagnostic Products Co., Los Angeles, CA). To eliminate interassay variance, all samples were analyzed within the same batch. All intra-assay variances were $<$ 4.9% (15).

Subjective rating of physical well-being. Various subjective ratings of physical well-being were monitored during the competition. At 6 p.m. of every sampling day, subjects recorded their rating of "muscle tension," "fatigue," "lightness of body," and "flexibility." These ratings were assessed using a scale of 1 (heavy) to 5 (light).

Profile of mood states (POMS). A Japanese version of the POMS questionnaire (17) was administrated at 6 p.m. on each sampling day. POMS scores were obtained for subjects on the state of "tension," "depression," "anger," "vigor," "fatigue," and "confusion." Each mood dimension was rated on a scale of 1 to 4 from "not at all" to "extremely." These subscales appeared to have at least face validity for measuring mood states among athletes. Morgan et al. (19) established the "iceberg profile," a specific POMS profile that was correlated with success in Olympic qualifying competitions. The "iceberg profile" is characterized by low levels of "tension," "depression," "anger," "fatigue," and "confusion," as well as high level of "vigor."

Data processing and statistical analysis. For analysis of SIgA levels, data were reported in two forms: (A) absolute concentration of SIgA ($\mu g \cdot mL^{-1}$) or (B) SIgA secretion rate ($\mu g \cdot min^{-1}$) or the total amount of SIgA in the saliva per unit time. SIgA secretion rate was calculated by multiplying absolute SIgA concentration ($\mu g \cdot mL^{-1}$) by saliva flow rate ($mL \cdot min^{-1}$), which was calculated by dividing the total volume of saliva obtained in each sample (mL) by the time taken to produce the saliva sample (minutes).

Salivary SIgA concentration, saliva flow rate, SIgA secretion rate, salivary cortisol, subjective ratings of physical well-being, and POMS score were analyzed separately by two-way ANOVA with repeated measures to determine the effect of treatment. When ANOVA showed significant effects, a Fisher's *post hoc* test was performed. For all analyses, a P-value equal or less than 0.05 was accepted as statistically significant.

RESULTS

Salivary SIgA levels. The SIgA secretion rate significantly decreased during and after 3 d of competition in the control group ($P < 0.05$) but did not decrease significantly in the treatment group (Fig. 3). The SIgA secretion rate was significantly higher in the acupuncture group than in the control group at days 1 and 2 ($P < 0.05$). The concentration of SIgA significantly decreased at day 1 of competition in the control group, but not in the treatment group. In contrast, the SIgA of the treatment group increased at day 3 (Fig. 4).

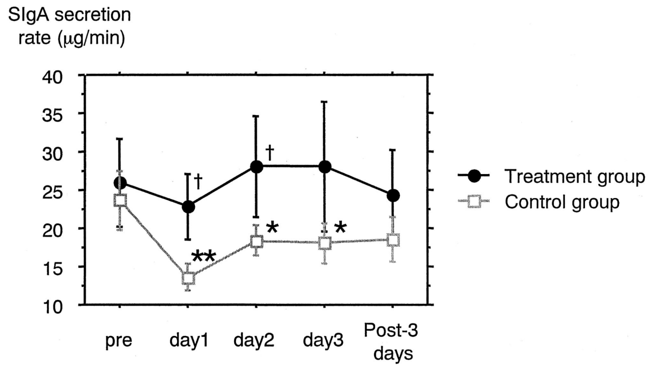

FIGURE 3—Change of SIgA secretion rate during the competitive period. SIgA secretion rate decreased at day 1 in competition and maintained in the control group. In the treatment group, SIgA secretion rate did not change. Data were expressed mean ± SE; * $P < 0.05$; ** $P < 0.01$ (vs pre); † $P < 0.05$ (between groups).

Salivary cortisol concentrations. The salivary concentration of cortisol significantly increased at days 2 and 3 in the control group ($P < 0.05$). In contrast, that in the acupuncture group increased at day 1 ($P < 0.05$) but returned to baseline at days 2 and 3 (Fig. 5). The salivary concentration of cortisol was significantly lower in acupuncture group than in the control group at days 2 and 3 ($P < 0.05$). The saliva flow rate and concentration of total protein did not change through the experimental period (data not shown).

Subjective rating of physical well-being. Compared with the control group, "muscle tension" was significantly lighter at days 1 and 2 in the acupuncture group ($P < 0.05$, Fig. 6). The score of "lightness of body" was significantly lower at day 3 in the control group than at baseline ($P < 0.05$) but was not significantly different from the acupuncture group. The score of "fatigue" did not change throughout the study in the acupuncture group, whereas it was heavier during the period of competition in the control group. Furthermore, the score at days 1 and 2 in the acupuncture group was lighter than that in the control group ($P < 0.05$, $P < 0.01$, respectively). "Flexibility"

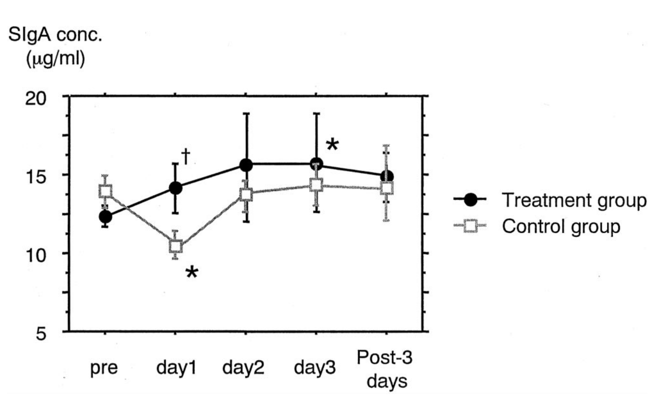

FIGURE 4—Change of SIgA concentration during the competitive period. SIgA concentration decreased at day 1 in the control group. In the treatment group, SIgA concentration did not change. Data were expressed mean ± SE; * $P < 0.05$ (vs pre); † $P < 0.05$ (between groups).

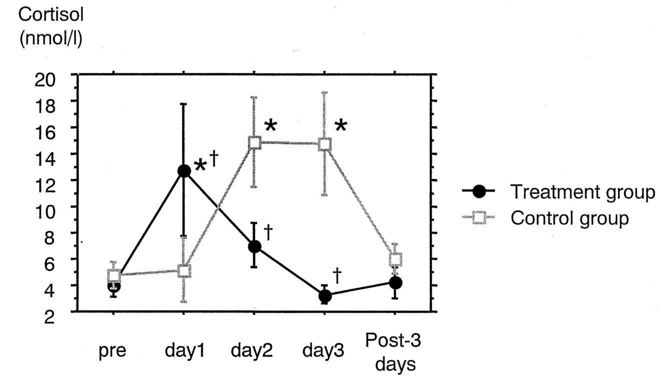

FIGURE 5—Change of salivary cortisol during the competitive period. Salivary concentration of cortisol increased at day 1 in competition and returned to baseline in the treatment group. In the control group, concentration of cortisol did not change at day 1 but increased at day 2 and day 3. Data were expressed mean ± SE; * $P < 0.05$; ** $P < 0.01$ (vs pre); † $P < 0.05$ (between groups).

score did not change significantly in both groups throughout the study.

POMS. In the acupuncture group, "fatigue" remained low during the period of physical competition, whereas the score at day 1 was lower than at baseline (pre, $P < 0.05$, Fig. 7). In the same group, "confusion" was lower after competition than at baseline ($P < 0.05$). In the control group, "fatigue" at days 1, 2, and 3 was higher than in the acupuncture group. The POMS score in day 3 was similar with that of day 2 (data not shown). These data suggest that mental fatigue in the control group was higher than in the acupuncture group during the period of sport activity. In contrast, the mental condition as reflected by the POMS showed an iceberg profile in the acupuncture group.

Total playing time in each group. The total playing time of each subject was given in Table 2. On day 2, the

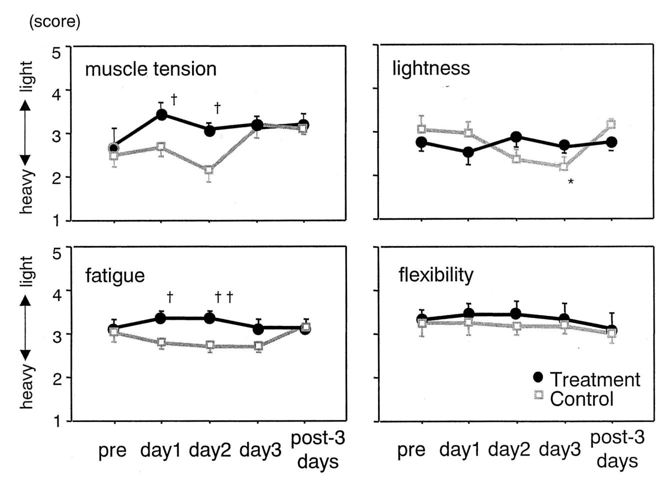

FIGURE 6—Changes of subjective rating of physical condition during the competitive period. Subjective ratings of muscle tension in the treatment group were lighter than that in the control group during the competition (day 1, day 2). Lightness score at day 3 in the control group was lower than the initial level. Subjective ratings of fatigue in the treatment group were lighter than that in the control group during the competition (day 1, day 2). Data were expressed mean ± SE; * $P < 0.05$ (vs pre); † $P < 0.05$; †† $P < 0.01$ (between groups).

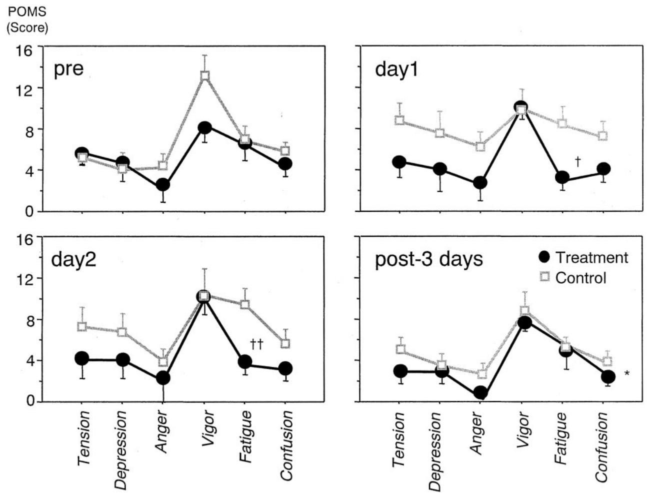

FIGURE 7—Changes of POMS score during the competitive period. POMS score in the treatment group maintained iceberg profile during the competition (day 1, day 2). Fatigue and score in the treatment group was lower than that in the control group during the competition. Data were expressed mean ± SE; * $P < 0.05$ (vs pre); † $P < 0.05$; †† $P < 0.01$ (between groups).

playing time in treatment group was longer than that in control group ($P < 0.05$).

DISCUSSION

Our study provides evidence that acupuncture treatment positively affects the salivary SIgA, salivary cortisol, subjective rating of physical well-being and POMS score in elite female soccer players during a relatively short period of sports competition. These results suggest that acupuncture treatment after physical exercise may improve the immunologic and endocrine states of elite female athletes. To our knowledge, the present data are the first to demonstrate that acupuncture treatment inhibits the reduction in mucosal immune function (salivary SIgA levels) induced by intense exercise, and modulates the stress hormone response to physical and competitive stress in elite female athletes.

Our study showed that exercise-induced decrease of salivary SIgA was inhibited in the acupuncture treatment group during the competition period. These data suggest that acupuncture treatment enhances SIgA secretion in the saliva during the period of continuous physical exercise. It is possible that the increased risk of URTI in athletes during the competition period is due to a decrease in SIgA levels. Thus, acupuncture treatment improves SIgA levels and such immunogenic action could lead to a reduced likelihood of infection in athletes and maintenance of physical well-being in female athletes. The exact mechanism of acupuncture-induced improvement of salivary SIgA secretion is not well understood. Sugiyama et al. (28) and Knardahl et al. (13) reported that acupuncture at ST 36 and LI 4 increased muscle sympathetic nerve activity, whereas heart rate, blood pressure, and skin perfusion remained unaffected. Salivary glands are controlled by both sympathetic and parasympathetic nervous systems. Thus, it is possible that acupuncture enhances SIgA secretion by stimulating the sympathetic nervous system. However, our results showed that the resting saliva flow rate did not change during the experimental period. Yang et al. (30) also reported that salivary SIgA increased at 24 h after acupuncture stimulation and continuous treatment of acupuncture significantly increased basal level of SIgA.

Competition differs from training by a greater degree of anxiety and stress that can amplify the hormonal variations usually recorded during exercise (22). Among the hormone reacting to exercise and stress, cortisol is probably the more affected. Increase of plasma as well as salivary cortisol levels during exercise is directly related to the intensity of exercise (5,11). Cortisol also reacts strongly to psychological stress, as during school examination (2). Previous studies demonstrated that intensified training and competition increase plasma cortisol concentration (26,29). In addition, other studies reported enhanced resting cortisol levels in overtrained athletes (12), although resting plasma cortisol levels in overtraining is controversial. Previous studies showed that salivary cortisol levels are correlated to plasma cortisol (27). In our study, the resting salivary cortisol levels increased at days 2 and 3 in the control group. However, acupuncture inhibited exercise-induced increase of salivary cortisol, although no such effect was noted at day 1. There, results suggest that continuous acupuncture treatment modulates the response of cortisol to intense exercise and competitive stress. Although Knardahl et al. (13) reported that plasma cortisol did not change after a single session of acupuncture treatment, competition-induced changes in salivary cortisol might be modulated by acupuncture treatment. A few reports have also shown that acupuncture treatment inhibited the increase of plasma cortisol after surgery (14). It is possible that acupuncture-related stimuli modulate stress response of cortisol.

In a number of studies, self-reported measures such as fatigue, muscle tension, quality of sleep, and other indicators of physical well-being have been monitored to assess adaptation of athletes during intense training and competition period. Miyamoto (18) reported that acupuncture treatment for athletes improved muscle tension and fatigue. In the present study, the acupuncture treatment group reported improvement in most of the subjective ratings of physical well-being, especially "muscle tension" and "fatigue" during the competition period, compared with the control group. Thus, acupuncture treatment improved the perception of physical well-being among elite soccer players.

Mood states have been shown to fluctuate with training volume and intensity (21). Previous studies suggested that mood states such as depression and anxiety may show changes with overtraining (19,21). Previous studies demonstrated significant changes in POMS measures with heavy or increased training (9). Elite athletes score below the general population average on all POMS variables expect vigor, in which they tend to score higher. In the present study, POMS scores suggested a higher mental fatigue in the control group than in the acupuncture group during the competition period. The fall of the fatigue score in the acupuncture group suggests that acupuncture treatment had good effects on mood states during the competition period. Mental well-being was stable in the treatment group showing an iceberg pattern of POMS. Morgan et al. (19) suggested that athletes who are successful exhibit a more pronounced iceberg profile (i.e., better emotional health) than do less successful athletes. Filaire et al. (6) also noted an iceberg profile during successful performance of the soccer team.

It is conceivable that other factors, such as fitness level, might influence the effects of acupuncture treatment on exercise capacity and perception of exertion. Furthermore, it should be recognized that acupuncture is generally practiced within a framework in which the therapy is individualized to the patients and the condition being treated. The present study design, by its very nature of uniformity, is inconsistent with this concept of therapeutic application and therefore may be limited in examining the effectiveness of acupuncture on an individual subject.

Filaire et al. (6) indicated that combined physiological and psychological changes during the training period are primarily of interest when monitoring training stress in relation to physical performance. Finally, studies relating hormonal variations during training and competition are still sparse, mainly because it is difficult to collect biological samples during official sports competition. Measurement of these components in saliva is easy to monitor immunological and hormonal changes to various situations including intense training and competition period.

In this study, the playing time on day 2 differed between groups. The treatment group demonstrated significantly lower cortisol and greater SIgA levels than the control group, although the treatment group played significantly longer than the control group. These data certainly support the positive influence of acupuncture treatment for the athletes of this study.

CONCLUSIONS

In the present study, we determined the effect of acupuncture treatment on physical and mental condition of elite female soccer players during sports competition. The following are the main results:

1. Exercise-induced fall in salivary SIgA level was inhibited in the acupuncture group during the competition period.
2. Exercise-induced increase in salivary cortisol was inhibited in the acupuncture group during the competition period.
3. Subjective rating of muscle tension and physical and mental fatigue diminished in the acupuncture group.
4. In the treatment group, POMS score showed an iceberg profile.

These data suggest that acupuncture treatment may improve the physical well-being of female athletes through changes in the immune and endocrine systems.

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