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# THE EFFECT OF A VOLLEYBALL PRACTICE ON ANABOLIC HORMONES AND INFLAMMATORY MARKERS IN ELITE MALE AND FEMALE ADOLESCENT PLAYERS

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## ABSTRACT

Eliakim, A, Portal, S, Zadik, Z, Rabinowitz, J, Adler-Portal, D, Cooper, DM, Zaldivar, F, and Nemet, D. The effect of a volleyball practice on anabolic hormones and inflammatory markers in elite male and female adolescent players. *J Strength Cond Res* 23(5): 1553–1559, 2009—The effect of a single exercise as well as exercise training on the growth hormone (GH)-insulin-like growth factor (IGF-I) axis and inflammatory cytokines was studied mainly in adults participating in individualized endurance-type sports. The gender-specific effect of exercise on these systems in adolescents is unknown. Therefore, the purpose of this study was to evaluate the effect of a typical volleyball practice on anabolic (GH, IGF-I, and testosterone) and catabolic hormones (cortisol) and inflammatory mediators (interleukin-6 [IL-6]) in elite, national team level, male ( $n = 14$ ) and female ( $n = 13$ ) adolescent volleyball players (13–18 years, Tanner stage 4–5). Exercise consisted of a typical 1-hour volleyball practice. Blood samples were collected before and immediately after the practice. Exercise led to significant increases in GH ( $0.2 \pm 0.1$  to  $2.7 \pm 0.7$  and  $1.7 \pm 0.5$  to  $6.4 \pm 1.4$  ng·mL<sup>-1</sup>, in men and women, respectively,  $p < 0.05$  for both), testosterone ( $6.1 \pm 0.9$  to  $7.3 \pm 1.0$  and  $2.4 \pm 0.6$  to  $3.3 \pm 0.7$  ng·mL<sup>-1</sup>, in men and women, respectively,  $p < 0.05$  for both), and IL-6 ( $1.1 \pm 0.6$  to  $3.1 \pm 1.5$  and  $1.2 \pm 0.5$  to  $2.5 \pm 1.1$  pg·mL<sup>-1</sup>, in men and women, respectively,  $p < 0.002$  for both). Exercise had no significant effect on IGF-I, insulin-like growth factor binding protein-3, and cortisol levels. There were no gender differences in the hormonal response to training.

Changes in GH and testosterone after the volleyball practice suggest exercise-related anabolic adaptations. The increase in IL-6 may indicate its important role in muscle tissue repair. These changes may serve as an objective quantitative tool to monitor training intensity in unique occasions in team sports.

**KEY WORDS** cytokines, gender, GH, IGF-I, training, team sport, youth athletes

## INTRODUCTION

The efficiency of exercise training depends on the training load and on the athlete's ability to tolerate it. Therefore, extensive efforts are made to quantify objectively the fine balance between training intensity and athlete's tolerance. Recent reports suggest that exercise leads to a simultaneous increase of antagonistic mediators. On the one hand, exercise stimulates anabolic components of the growth hormone (GH) → insulin-like growth factor-I (IGF-I) axis (1,7,27), and on the other hand, exercise elevates catabolic proinflammatory cytokines such as interleukin-6 (IL-6), IL-1, and tumor necrosis factor- $\alpha$  (20,24). Thus, it was suggested that evaluation of the exercise-induced changes in these circulating mediators can provide a new insight into quantification of training loads.

Previous studies focused on adults and on individualized sports and demonstrated, mainly, the effects of endurance-type and resistance exercise bouts and/or training on the GH-IGF-I axis and on inflammatory cytokines (20,22,24). Only recently, several studies examined the effect of supramaximal anaerobic bout (Wingate anaerobic test) and sprint interval training on these mediators (19,30). Very few studies have examined the effect of training on the GH-IGF-I axis and inflammatory markers in children and adolescent elite athletes and in team sports, which are very popular in these age groups (21). Moreover, gender-associated differences

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in the response of these circulating mediators to exercise training in elite athletes have not been studied thoroughly.

Therefore, the aim of the present study was to evaluate the effect of a common single volleyball practice on the balance between anabolic and catabolic hormones and circulating cytokines in a group of elite, national team level, male and female volleyball players. In addition, we compared the gender-specific exercise-related response. We hypothesized that, as seen previously in individualized sports, volleyball practice will lead to a significant increase in both anabolic and inflammatory mediators.

## METHODS

### Experimental Approach to the Problem

The effect of a single exercise and/or exercise training on the GH-IGF-I axis and on inflammatory cytokines was studied mainly in adult athletes and in individualized endurance-type sports. Moreover, the gender-specific effect of exercise on these systems in adolescents is unknown. Therefore, the purpose of the present study was to evaluate the effect of a typical single volleyball practice on anabolic and catabolic hormones and on inflammatory mediators in elite, national team level, male and female, late pubertal, volleyball players. Due to the relatively large number of male ( $n = 14$ ) and female ( $n = 13$ ) elite players, we were able to compare the exercise-induced response in both genders. We chose volleyball, a very popular team sport for both genders, which involves both aerobic and anaerobic properties. Hormonal measurements included circulating levels of the anabolic hormones GH, IGF-I, insulin-like growth factor binding protein-3 (IGFBP-3), and testosterone and the catabolic hormone cortisol. Measurements of inflammatory mediators included the proinflammatory markers, IL-6, and the anti-inflammatory marker, interleukin-1 receptor antagonist (IL-1ra). In addition, we measured serum lactate levels, a commonly used marker for the assessment of training intensity.

### Subjects

Twenty-seven (14 men and 13 women), healthy elite, national team level, Israeli junior volleyball players (age range 13.5-18 years, Tanner stage for pubic hair 4-5) participated in the study. All participants played in the Israeli Premier Junior Volleyball League, belonged to the Israeli national junior volleyball team, and were members of the Israeli National Academy for Gifted Athletes. The study was performed during the very early phase of the volleyball season. The study was approved by the local

institutional review board. All parents and participants signed an informed consent before participation in the study.

Participants trained for 18 to 22 hours per week. Training involved mainly tactic and technical drills emphasizing volleyball skills and team strategies, jumping and speed drills with and without the ball, and longer interval sessions of hits and digs (e.g., several repetitions of 30-60 seconds of quick hits over the net with sprinting from the net to the end of the court in between). No resistance training was done at the time of the study.

Anthropometric and fitness characteristics of the participants are summarized in Table 1. Standard calibrated scales and stadiometer were used to determine height, body mass, and body mass index. Skinfold measurements at 4 sites (triceps, biceps, subscapular, and suprailiac) were used to calculate percent body fat using standard equations (18).

### Procedure

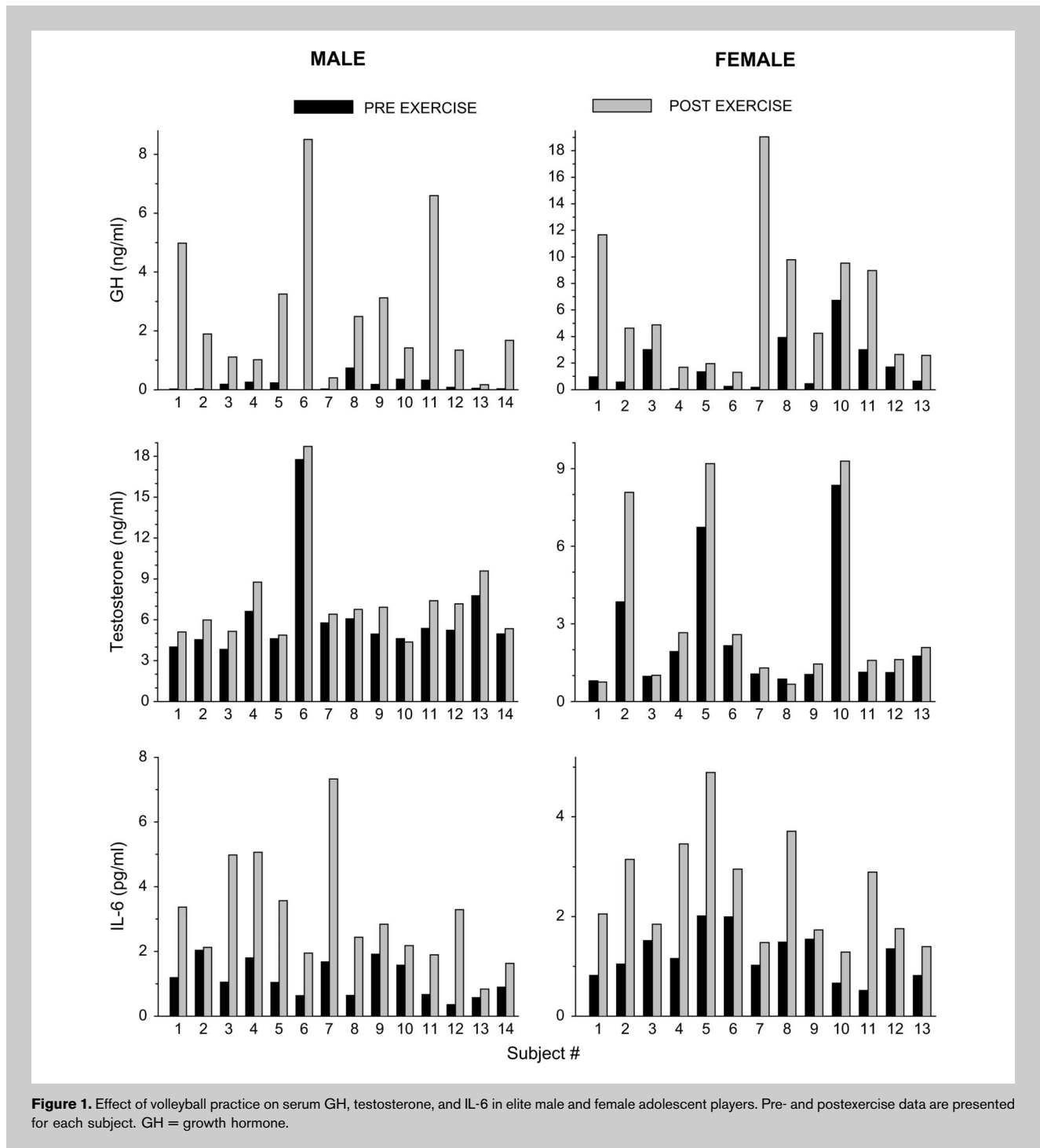
**Exercise Protocol.** Each participant performed a typical 1-hour morning volleyball practice at fasting state (i.e., 7 AM to 8 AM). Training consisted of 20-minute dynamic warm-up, which included jogging, stretching, and running drills at submaximal speed (up to 80% of maximal speed), and an additional 20 minutes of volleyball drills. The main part of the practice included 7 repetitions of 7 consecutive sprints from the back of the volleyball court to the net, maximal jump, and a hit of the volleyball over the net at the end of each sprint. Each repetition lasted about 1.5 minutes with 1-minute rest to collect the balls between repetitions. Participants did not train during the day before the study.

**Blood Sampling and Analysis.** Blood samples were collected immediately before and after the training session. All female participants had regular menses, and blood samples were collected during the early follicular phase of their menstrual cycle (first 5 days of the cycle). Blood samples were immediately spun at 3,000 rpm, at 4° C for 20 minutes. The serum was separated and stored at -80° C. All pre- and postexercise specimens from each individual were analyzed in

**TABLE 1.** Anthropometric and fitness characteristics of the study participants.

	Men ( $n = 14$ )	Women ( $n = 13$ )
Age (y)	16.3 ± 0.3	16.0 ± 0.4
Height (cm)	190.9 ± 1.2	175.6 ± 1.8*
Weight (kg)	77.4 ± 1.4	64.1 ± 1.8*
BMI ( $\text{kg}\cdot\text{m}^{-2}$ )	21.2 ± 0.6	20.8 ± 0.6
Fat (%)	13.5 ± 1.1	24.2 ± 0.9*
Tanner stage	4.5 ± 0.1	4.3 ± 0.2
VO <sub>2</sub> max ( $\text{ml}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ )	41.2 ± 1.5	42.0 ± 1.8
Anaerobic ( $\text{W}\cdot\text{kg}^{-1}$ )	9.3 ± 0.2	8.2 ± 0.3*

\* $P < 0.05$ .



the same batch by an experienced technician who was blinded to gender and order of samples.

**Growth Hormone.** Growth hormone serum concentrations were determined by enzyme-linked immunosorbent assay (ELISA) with the use of the DSL-10-1900 Active kit

(Diagnostic System Laboratories, Webster, TX). Intra-assay coefficient of variation (CV) was 3.3 to 4.5%, interassay CV was 5.5 to 12.9%, and the sensitivity was 0.03 ng·mL<sup>-1</sup>.

**Insulin-Like Growth Factor-I.** Insulin-like growth factor-I was extracted from IGF-BPs by using the acid-ethanol extraction

method. Serum IGF-I concentrations were determined by a 2-site immunoradiometric assay by using the DSL-5600 Active kit (Diagnostic System Laboratories). IGF-I intra-assay CV was 1.5 to 3.4% and the interassay CV was 3.7 to 8.2%. Assay sensitivity was 0.8 ng·mL<sup>-1</sup>.

**Insulin-Like Growth Factor Binding Protein-3.** Insulin-like growth factor binding protein-3 serum concentrations were determined by ELISA with the use of the DSL-10-6600 Active kit (Diagnostic System Laboratories). Intra-assay CV was 7.3 to 9.6%, interassay CV was 8.2 to 11.4%, and the sensitivity was 0.04 ng·mL<sup>-1</sup>.

**Lactate.** Serum lactate was measured spectrophotometrically (YSI 1500; YSI Inc., Yellow Springs, OH). Intra-assay CV was 2.8%, interassay CV was 3.5%, and the sensitivity was 0.2 mmol·L<sup>-1</sup>.

**Cortisol.** Serum cortisol levels were determined by a commercial radioimmunoassay (Diagnostic Products Corporation, Los Angeles, CA). The intra- and interassay CVs for this assay were 3.2 and 6.8%, respectively.

**Testosterone.** Testosterone serum concentrations were determined by ELISA with the use of the DSL commercial kit (Diagnostic System Laboratories). Intra-assay CV was 4.8 to 5.3%, interassay CV was 2.8 to 4.9%, and the sensitivity was 0.04 ng·mL<sup>-1</sup>.

**Inflammatory Mediators.** Inflammatory mediators were analyzed by ELISA, using the R&D System Quantikine High Sensitivity commercial kits (R&D Systems, Minneapolis, MN). Interleukin-6: intra-assay CV was 3.8 to 11.1%, interassay CV was 7.1 to 29.5%, and the sensitivity was 0.009 pg·mL<sup>-1</sup>. Interleukin-1 receptor antagonist: intra-assay CV

was 3.1 to 6.2%, interassay CV was 4.4 to 6.7%, and the sensitivity was 22 pg·mL<sup>-1</sup>.

### Statistical Analyses

Two-sample *t*-test was used to compare baseline and postexercise anthropometric fitness and hormonal levels between male and female players. Repeated measures analysis of variance was used to assess the effect of exercise on circulating components of the GH-IGF-I axis and inflammatory mediators with time serving as the within-group factor and gender as the between-group factor. Data are presented as mean ± SEM. Significance was set at an alpha level of  $p \leq 0.05$ .

### RESULTS

Individual changes in GH, testosterone, and IL-6 are presented in Figure 1.

The gender-specific effect of the training session on anabolic and catabolic hormones and on inflammatory mediators is summarized in Table 2 and Figure 2. Training led to significant increases in the anabolic hormones GH and testosterone and in the proinflammatory mediator IL-6 in both groups. There was no significant training-induced effect on IGF-I, IGFBP-3, cortisol, and IL-1ra in either the male or the female volleyball players.

In the female players, baseline levels of GH and cortisol were significantly higher and testosterone levels significantly lower compared with male players. Levels of testosterone were significantly lower in the female players at the end of exercise as well. There was no significant difference in the volleyball practice-induced changes in GH, testosterone, and IL-6 levels between genders.

**TABLE 2.** Gender differences in the anabolic/catabolic and inflammatory response to a single training in elite adolescent volleyball players.‡§

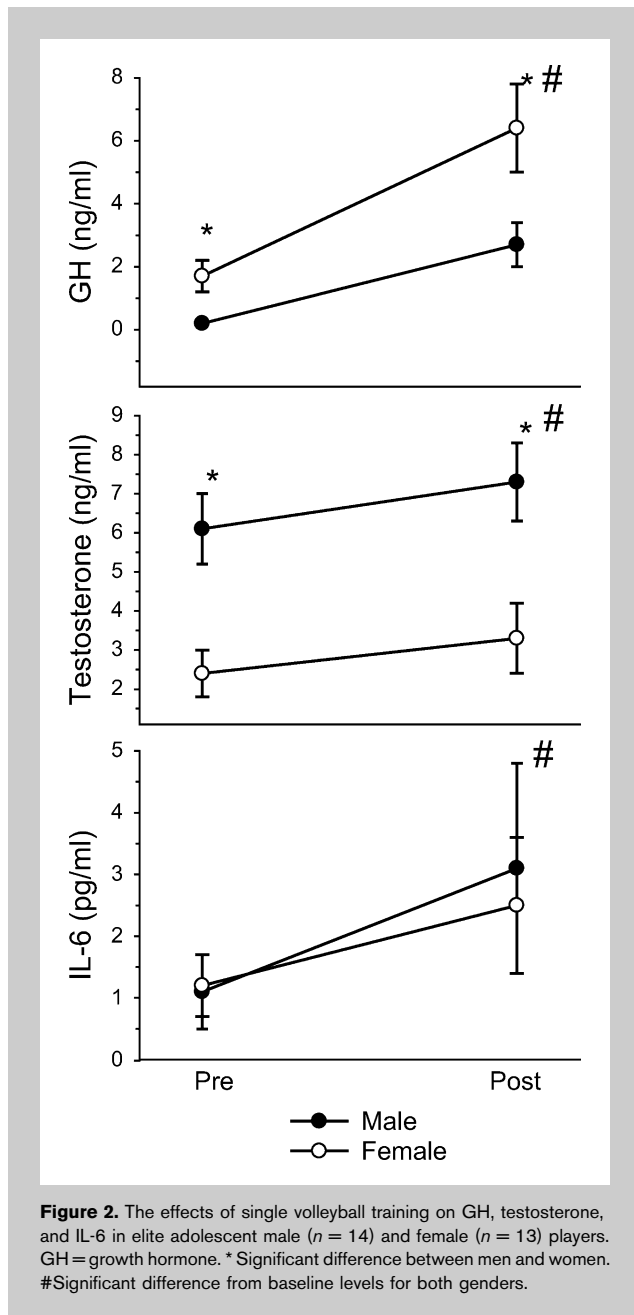
	Men ( <i>n</i> = 14)		Women ( <i>n</i> = 13)	
	Pre	Post	Pre	Post
Lactate (mmol·L <sup>-1</sup> )	3.1 ± 0.2	5.7 ± 0.5*	3.0 ± 0.2	5.1 ± 0.3*
IGF-I (ng·mL <sup>-1</sup> )	506.7 ± 33.6	522.3 ± 25.8	519.8 ± 24.7	537.7 ± 17.7
IGFBP-3 (ng·mL <sup>-1</sup> )	6,025.6 ± 235.9	5,986.7 ± 364.0	6,121.6 ± 263.5	6,268.8 ± 214.3
Cortisol (µg·L <sup>-1</sup> )	20.8 ± 1.8	22.1 ± 1.6	28.4 ± 2.7†	32.6 ± 4.8†
IL-1ra (pg·mL <sup>-1</sup> )	296.4 ± 22.9	319.8 ± 23.4	360.8 ± 31.2	381.0 ± 40.5

\*Statistically significant difference from baseline.

†Statistically significant gender differences.

‡IGF-I = insulin-like growth factor-I; IGFBP-3 = insulin-like growth factor binding protein-3; IL-1ra = interleukin-1 receptor antagonist.

§Data are presented as mean ± SEM.



## DISCUSSION

The effect of a single exercise and/or exercise training on anabolic and catabolic hormones and on inflammatory cytokines was studied mainly in adults and in individualized resistance and endurance-type sports. Very few studies examined the effect of anaerobic-type sports and team sports training on these systems (9,19,21,30). In the present study, we examined the effect of a single typical volleyball practice, a popular sport among adolescents involving both aerobic and anaerobic properties at mild to moderate intensities, on anabolic and catabolic hormones and circulating pro- and

anti-inflammatory mediators in elite male and female volleyball players. Exercise was associated with a significant increase in the anabolic hormones GH and testosterone. In addition, exercise led to a significant increase in the pro-inflammatory cytokine IL-6. Exercise had no significant effect on IGF-I, IGFBP-3, cortisol, and IL-1ra levels. There were no significant gender-related differences in the hormonal and inflammatory responses to the volleyball practice.

Previous studies that examined the effects of endurance-type and anaerobic-type exercise on GH suggested that the exercise input should be sufficient to cause a sizeable metabolic effect (e.g., above the lactic anaerobic threshold) to stimulate GH secretion (8). Interestingly, we found that even a moderate volleyball training (mean end-exercise lactate level  $5.4 \text{ mmol}\cdot\text{L}^{-1}$ ) led to a significant increase in GH levels. Moreover, previous studies indicated that the exercise-induced GH peak occurs 25 to 35 minutes after the start of exercise irrespective of the exercise duration and occurs a few minutes earlier in women (6,27,30,33). Therefore, because blood samples were collected in the present study only before and at the end of exercise (1 hour), and not 25 to 30 minutes after the beginning of exercise, it is possible that the exercise-related GH peak might have been even higher if these blood samples were drawn earlier.

Baseline and postexercise GH levels were significantly higher in women compared with men. However, the response to training was not significantly different between genders. Consistent with our findings, previous reports demonstrated that GH levels are higher in women than in men of comparable age (13,26). Growth hormone secretion exhibits a circadian rhythm in both genders with the highest secretion rates during periods of deep sleep in the first hours of the night (midnight to 3 am) (14). However, whereas GH secretion occurs mainly at night and is significantly reduced during the rest of the day in men, GH secretion is much more uniform throughout the day in women (14,29). Moreover, GH levels were found to correlate positively with estrogen levels in women (14). Interestingly, we did not find gender differences in the GH response to training. However, it should be emphasized again that the blood samples were collected at the end of the exercise training and probably not during the peak GH secretion.

Baseline and postexercise testosterone levels were significantly higher in men compared with women. However, training was associated with an increase in testosterone levels in both genders, and the response to training was not significantly different between genders. The testosterone increase may indicate an exercise-associated anabolic adaptation. Although the effects of different types of exercise on testosterone levels in men are very well studied, few previous studies examined the effect of resistance and endurance exercise on testosterone level in women (e.g., (3,31)). Circulating levels of testosterone have been shown to increase in response to acute bout of endurance exercise in women across a wide age range (from young adult to

premenopausal (2–4)). In contrast, the effect of resistance training on circulating testosterone levels was less consistent. Several studies demonstrated an increase of 16 to 25% in testosterone levels after resistance exercise in women (5,23,32). Other studies have failed to demonstrate an increase in testosterone levels in women at different stages of the menstrual cycle (15,17). Very few studies examined the effect of team sports training on testosterone levels in female athletes. In contrast to our findings, there were no significant changes in circulating testosterone and salivary testosterone levels in elite female players after an intense water polo practice and handball match, respectively (9,12).

In contrast to male athletes, the source of the exercise-induced testosterone production in female athletes is the adrenal gland and obviously not the testicles. Accordingly, postexercise increases in testosterone levels in female athletes were usually accompanied by a parallel increase in cortisol, dehydroepiandrosterone, and/or androstenedione levels (3,5,12). Interestingly, in the present study, the volleyball practice did not lead to a significant increase in cortisol levels. However, there was a significant correlation between the exercise-induced changes in testosterone and cortisol ( $r = 0.53$ ,  $p < 0.01$ ) in the adolescent female volleyball players. Finally, despite the significantly lower testosterone levels in the female players (baseline and post exercise), there was no significant difference in the response to exercise between genders. The results suggest, therefore, that increase in testosterone levels may play an important role in the anabolic response to exercise in female athletes as well. In addition, the parallel exercise-related increase in GH and testosterone suggests a possible mechanistic link for the exercise-associated stimulation, secretion, or release of these hormones.

The exercise had no effect on circulating IGF-I and IGFBP-3 levels. IGF-I plays a central role in the exercise-induced muscle adaptation (1). Previous reports suggested that very short supramaximal exercise efforts (e.g., 90 seconds) lead to increases in IGF-I levels (16) and intense sprint interval training increased IGFBP-3 levels (19). It is possible, therefore, that the present volleyball practice was not intense enough to increase IGF-I and/or IGFBP-3 levels.

The volleyball training was also associated with an increase in IL-6 levels. There was no significant change in the anti-inflammatory mediator IL-1ra after the training. Previous studies found increases in IL-6 and IL-1ra after intense, prolonged, endurance-type exercise sessions (20,22,24,28). Therefore, our finding of an increase of only IL-6 after relatively moderate intensity volleyball training suggests, probably, that IL-6 is the most sensitive inflammatory cytokine to exercise.

The major source for the exercise-related IL-6 increase is the skeletal muscle (10). Interleukin-6 increases during exercise both with and without evidence of muscle damage. However, IL-6 is believed to play an important mediatory role in the inflammatory response needed for the exercise-

associated muscle damage repair (25,28). It is possible that frequent jumping and/or ball hitting and digging during the typical volleyball practice were associated with subclinical muscular and/or soft tissue damage that triggered a significant increase in circulating IL-6 levels. In addition, it was previously demonstrated that IL-6 may alter IGF-I activity through a variety of mechanisms including direct inhibition of IGF-I production (11). It is possible, therefore, that the exercise-related increase in IL-6 prevented the increase in IGF-I after the volleyball training.

## PRACTICAL APPLICATIONS

The present study examined the hormonal and inflammatory responses to a single typical volleyball practice. Changes in the GH and testosterone suggested mainly exercise-related anabolic adaptations, and increases of IL-6 may indicate its important role in muscle tissue repair after volleyball training. There were no significant gender-related differences in the hormonal and inflammatory responses to the volleyball practice, suggesting that even testosterone, despite the low basal and postexercise levels in female athletes, can be used as an exercise-associated anabolic marker. The results indicate that changes in the anabolic-catabolic hormonal balance and in circulating inflammatory cytokines can be used by the athlete and/or his coach to gauge the training intensity also in team sports such as volleyball. It is clear that these responses cannot be used as a marker for every practice, unless future techniques provide immediate results (like the current ability to assess lactate levels). However, the response of these hormones can be used occasionally in different types of team sports, important training sessions, or training camps or before main competitions or tournaments as an objective quantitative tool to monitor training load and to better plan training cycles throughout the competitive season.

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