INTRODUCTION

The region between the ventilatory threshold (VT) and respiratory compensation point (RCP) is defined as the isocapnic buffering (ICB) phase and represents a phase of compensation for exercise-induced metabolic acidosis. There is sparse literature examining the effects of physical training on ICB phase in athletes.

Objectives: The purpose of this study was to examine the effects of a repeated sprint training program on the ICB phase of college volleyball players.

Methods: Eighteen male volleyball players were randomly assigned to either an experimental group (n=9) or a control group (n=9) and followed a traditional volleyball training program three times per week for six weeks. The experimental group additionally performed a repeated sprint training protocol immediately before each volleyball training session. Before and after the 6-week training period, all participants performed an incremental treadmill test to determine VT, RCP, and maximal oxygen uptake (VO2max).

The ICB phases were calculated as VO2 (ml/kg/min) and sprint speed (km/h).

RESULTS

The experimental group showed significant improvements in ICB phase, RCP, VO2max and maximal sprint speed after training (p<0.01). There were no significant changes in VT after training in the experimental group (p>0.05). None of these variables changed significantly in the control group (p>0.05).

Conclusions: These findings indicate that repeated sprint training can enhance the ICB phase of volleyball players, which may be attributable to an improvement in buffering capacity leading to a shift in RCP towards higher intensities without any change in VT. The increase in the ICB phase may play an important factor in terms of improvement in the high-intensity exercise tolerance of athletes. Level of Evidence II, Therapeutic studies - Investigating the results of treatment.

Keywords: Oxygen consumption; Anaerobic threshold; Hyperventilation; Acidosis.
INTRODUCTION

During incremental exercise, anaerobic threshold (AT) can be determined from non-invasive gas exchange measurements alternative to the measurements of blood lactate concentration, in this case referred to as the ventilatory threshold (VT). VT corresponds to the nonlinear increase in carbon dioxide production and ventilation due to the bicarbonate buffering of hydrogen ions (H+) in response to the systematic increase of blood lactate above resting values. When H+ can no longer be compensated by circulating bicarbonate leads to a decrease in blood pH and stimulates the carotid bodies to increase ventilatory drive results in hyperventilation. This additional ventilatory response is called the respiratory compensation point (RCP). The region between AT and RC is defined as hypocapnic hyperventilation (HHV). A region between RCP and the end of exercise is defined as the phase of hypocapnic hyperventilation (HHV).

A few researchers investigated effects of physical training on ICB phase in the endurance athletes; however, showed different results. Consistent with many studies, we recently reported that the addition of a repeated sprint training (RST) program to normal volleyball training sessions can improve both the VO2max and high-intensity intermittent exercise performance of college volleyball players. However, to our knowledge, there is no previous study examining the effects of RST on the ICB phase during incremental exercise. The purpose of this study was to examine the effects of a RST program on the ICB phase during incremental exercise in college volleyball players.

METHODS

Eighteen male volleyball players competing in Division I of the Turkish University League volunteered to participate in the study. All of the subjects were members of the Erciyes University volleyball team. All players had trained and competed regularly in volleyball for at least 4 years. The Erciyes University Ethics Committee approved the study (2016/651). All testing and training procedures were fully explained, and written informed consent was obtained for each subject. During the study, the players were not allowed to perform any additional conditioning training that would affect the results of the study.

The experimental protocol consisted of baseline testing, a 6-week training intervention, and post-testing. After baseline testing, 18 volleyball players were randomly divided into two equal groups: the experimental (EXG; mean ± SD; age 21.2 ± 1.3 years, height 183.4 ± 5.4 cm, weight 71.1 ± 7.3 kg) and control groups (CG; age 21.2 ± 1.6 years, height 184 ± 4.4 cm, weight 75.7 ± 8.5 kg). Both groups followed a traditional volleyball training program three times per week for 6 weeks. All training sessions in both groups were conducted at the same time of day on Monday, Wednesday and Friday of each consecutive week. The experimental group additionally performed RST prior to their normal volleyball training sessions.

Maximal oxygen uptake (VO2max), VT and RCP were determined from a progressive intensity and continuous effort treadmill protocol. All tests were performed on a motorized treadmill (h/p/Cosmos Quasar med, Nussdorf-Traunstein, Germany). VO2, VCO2 and VE were measured online using a breath-by-breath cardiopulmonary exercise testing system (Quark PFT Ergo, CosmedSrl, Rome, Italy). Breath-by-breath data was smoothed using a five-step average filter and then reduced to 15 s stationary averages.

Players started running at 7 km/h with speed increments of 1 km/h every minute until they could no longer keep pace. The VO2max was defined as the highest 15 s VO2 value reached during the incremental test and expressed as a relative value (milliliters per minute per body mass; ml/kg/min). Achievement of VO2max was considered as the attainment of at least two of the following criteria: 1) a plateau in VO2 despite increasing speed, 2) a respiratory exchange ratio (VCO2/VO2) above 1.10, and 3) a HR (heart rate) within 10 beats per minute of age-predicted maximum HR (220 – age). Time to exhaustion was recorded as the time from the start of the run until the point of exhaustion. Maximal respiratory exchange ratio (RERmax) was expressed as the highest 15 s average value obtained during the last stage of the incremental exercise test.

The VT and RCP were determined using the V-slope method described by Beaver et al. The VT and RCP were defined as the VO2 value corresponding to the intersection of two linear regression lines derived separately from the data points below and above the breakpoint in the VCO2 versus VO2, and VE versus VCO2 relationships, respectively (Figure 1). Additionally, to increase the accuracy of the identification of VT and RCP, a visual identification technique was used as described below. VT was determined using the criteria of an increase in VE/VO2 with no increase in VE/VCO2 and an increase in end-tidal O2 pressure with no fall in end-tidal CO2 pressure, whereas RCP corresponded to an increase in VE/VCO2 and decrease in end-tidal CO2 pressure. To reduce the variability connected with the identification of VT and RCP, analyses were performed by two independent investigators. If there was disagreement, the opinion of a third investigator was sought. Each of the following variables was recorded at both the VT and the RC: running speed (km/h), VO2 (ml/kg/min) and VCO2 as a percentage of VO2max (%VO2max). Linear regression analyses

Palabras Clave: Consumo de oxígeno; Umbral anaeróbico; Hiperventilación; Acidosis.
were performed by using the Sigma Plot program (Sigma Plot 12.0, Systat Software Inc., Chicago, USA).

ICB phase was calculated as the difference in VO₂ (ICB_VO₂) and running speed (ICB_SPEED) between RCP and VT, and expressed in either absolute or relative values (expressed as a percentage of RCP previously described by Rocker et al.).

HHV phase was calculated as the difference in VO₂ (HHV_VO₂) and running speed (HHV_SPEED) between the end of exercise and RCP, and expressed in either absolute or relative values (expressed as a percentage of VO₂max and maximal running speed).

During the six-week training period, the players in both groups continued their regular volleyball training program. Volleyball training for the development of technical and tactical skills was the same in both groups. The main part of the volleyball session included serving, passing, and setting in small groups, blocking and spiking technique, small-sided games to work on offensive and defensive strategies, and individual tactics.

The RST was performed three times per week immediately before the volleyball training session. Details of the weekly RST program are given in Table 1. The RST consisted of 1–3 sets of 3–5 × 20-m maximal sprints with 20 s of active recovery between sprints and 4 min of passive recovery between sets. Each sprint represents a maximal effort with 20 s allowed between each sprint for the turnaround. The recovery time was controlled by a hand-held stopwatch. Training volume was progressively increased over 6 weeks.

### Statistical analysis

Data are reported as means ± standard deviation (SD). Statistical significance was accepted at p<0.05. The normality of the data was examined by assessing the Shapiro-Wilk test on all measured variables. The differences in all baseline measures between EXG and CG were evaluated by the unpaired t-test for normally distributed data, and Wilcoxon matched-pair signed-rank test for non-normally distributed data.

### RESULTS

There were no significant differences between the two groups for age, height, body mass and the baseline physiological variables (p > 0.05, Table 2). After the training period maximal running speed, the time to exhaustion, VO₂max and RER max during the incremental treadmill test were increased in the EXG (p < 0.01, Table 2). There were no significant changes in values of VO₂ and running speeds corresponding to VT and VT expressed as %VO₂max after training in the experimental group (p > 0.05). The EXG showed a significant increase in values of VO₂ and running speeds corresponding to RCP, and RCP expressed as %VO₂max after training (p < 0.01).

### Table 1. The 6 weeks of RST program, showing the number of sets and repetitions for each session.

<table>
<thead>
<tr>
<th>Week</th>
<th>Session</th>
<th>Number of sets</th>
<th>Repetitions × Distance (m)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1</td>
<td>1</td>
<td>5 × 20</td>
</tr>
<tr>
<td>2</td>
<td>2</td>
<td>1</td>
<td>5 × 20</td>
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<tr>
<td>3</td>
<td>3</td>
<td>1</td>
<td>5 × 20</td>
</tr>
<tr>
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<td>4</td>
<td>1</td>
<td>5 × 20</td>
</tr>
<tr>
<td>5</td>
<td>5</td>
<td>1</td>
<td>5 × 20</td>
</tr>
<tr>
<td>6</td>
<td>6</td>
<td>1</td>
<td>5 × 20</td>
</tr>
</tbody>
</table>

### Table 2. Physiological variables corresponding to the ventilator threshold, respiratory compensation point, maximal values, isocapnic buffering and hypocapnic hyperventilation phases of the experimental and control groups baseline and after the 6 weeks training period.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Experimental Group (n = 9)</th>
<th>Control Group (n = 9)</th>
</tr>
</thead>
<tbody>
<tr>
<td>VO₂max (l/min)</td>
<td>37.7 ± 5</td>
<td>39.6 ± 5.5</td>
</tr>
<tr>
<td>VO₂max (%)</td>
<td>75.2 ± 6.9</td>
<td>74 ± 9</td>
</tr>
<tr>
<td>SpeedVT (m/s)</td>
<td>10.7 ± 1.4</td>
<td>11 ± 1.6</td>
</tr>
<tr>
<td>VO₂VT (l/min)</td>
<td>47.8 ± 3.4*</td>
<td>44.7 ± 4.7</td>
</tr>
<tr>
<td>RCP%VO₂max</td>
<td>85.7 ± 4.8</td>
<td>89.3 ± 4.8*</td>
</tr>
<tr>
<td>SpeedRCP (m/s)</td>
<td>13.1 ± 1.3</td>
<td>13.2 ± 1.4</td>
</tr>
<tr>
<td>VO₂RCP (l/min)</td>
<td>50 ± 3.6</td>
<td>53.4 ± 1.8*</td>
</tr>
<tr>
<td>SpeedVT (%)</td>
<td>15.6 ± 1.3</td>
<td>16.8 ± 1*</td>
</tr>
<tr>
<td>Time to exh. (min)</td>
<td>8.93 ± 1.3</td>
<td>10.27 ± 1.1</td>
</tr>
<tr>
<td>RERmax</td>
<td>1.16 ± 0.4</td>
<td>1.23 ± 0.4</td>
</tr>
<tr>
<td>AbsICB_VO₂</td>
<td>5.2 ± 2.2</td>
<td>8 ± 3.1*</td>
</tr>
<tr>
<td>AbsICB_SPEED</td>
<td>12.2 ± 4.7</td>
<td>17.2 ± 6.9*</td>
</tr>
<tr>
<td>AbsICB_SPEED (%)</td>
<td>2.3 ± 0.5</td>
<td>3.6 ± 1*</td>
</tr>
<tr>
<td>AbsRCP_VO₂</td>
<td>17.9 ± 4.2</td>
<td>25 ± 6.7*</td>
</tr>
<tr>
<td>AbsRCP_SPEED</td>
<td>7.2 ± 2</td>
<td>5.6 ± 2.4*</td>
</tr>
<tr>
<td>AbsHHV_VO₂</td>
<td>14.2 ± 4.8</td>
<td>10.6 ± 4.8*</td>
</tr>
<tr>
<td>AbsHHV_SPEED</td>
<td>2.5 ± 0.8</td>
<td>2.2 ± 0.6</td>
</tr>
<tr>
<td>AbsICB%VO₂</td>
<td>16.2 ± 5.2</td>
<td>15.2 ± 6.9</td>
</tr>
</tbody>
</table>

Values are mean ± standard deviation VO₂ and running speed are expressed in ml min⁻¹ and km h⁻¹, respectively. * Significantly different from pre-training (baseline) values. VT = Ventilator threshold, RCP = Respiratory compensation point, Time to ex = Time to exhaustion (min), VO₂max = maximal oxygen uptake, RERmax = maximal respiratory exchange ratio, Abs = absolute, Rel = relative, Speedmax = maximal running speed, ICB = Isocapnic buffering; HHV = Hypocapnic hyperventilation.

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**Figure 1.** Examples showing methods of determining the ventilatory threshold (A) and the respiratory compensation point (B) in one subject.
Both absolute and relative values of ICB<sub>VO2</sub> and ICB<sub>SPEED</sub> were significantly increased in the EXG after training (p<0.01). The both absolute and relative HHV<sub>VO2</sub> were significantly reduced in the EXG (p<0.05), while the absolute and relative HHV<sub>SPEED</sub> were unchanged after 6-week training (p>0.05). None of these variables were significantly changed in the CG (p>0.05).

DISCUSSION

The results of this study indicated that the RST program led to a significant improvement in ICB phase, RCP and VO<sub>2max</sub> in the EXG while no change occurred in the VT. None of these variables were significantly changed in the CG during the training program, suggesting that volleyball training alone may not suffice for the improvement of buffering capacity. Our findings suggest that RST might have improved the buffering capacity leading to a shift of RCP to higher intensities and improve high-intensity exercise tolerance, and consequently enhancing the ICB phase.

No significant change was observed in the VT of the EXG, whereas improved in RCP and VO<sub>2max</sub>. Consistent with our results, previous studies have shown that no significant change in AT after maximal sprint interval training, whereas improve in VO<sub>2max</sub> in VO<sub>2max</sub> and AT. In contrast, some studies have demonstrated that AT may improve without changes in VO<sub>2max</sub> after the endurance training and over the football season. Although both VO<sub>2max</sub> and AT are the most important physiological variables associated with aerobic performance, the two variables appear to have different physiological mechanisms. It has been suggested that lower intensity training at slightly below AT improves AT and induces mainly central adaptations such as improvements in pulmonary diffusion, hemoglobin affinity, and increases in cardiac output. On the other hand, higher intensity training at VO<sub>2max</sub> induces mainly peripheral adaptations, which provide the improvement of VO<sub>2max</sub> such as increases in oxidative enzyme activity, mitochondrial volume and density, and myoglobin. It has been shown that sprint interval training induces specific metabolic adaptations leading to increase in VO<sub>2max</sub>. Our findings suggest that RST can be effective in enhancing VO<sub>2max</sub> but not AT; this may have been due to the maximal intensity of the training sessions.

Our findings demonstrate that the repeated sprint training represents an effective means of increasing RC rather than VT. Oshima at al. has shown that endurance training above the AT more effective in increasing the RCP than in increasing VT. The longer ICB phase observed in EXG after training may associate with shift of RCP to higher intensities. RST sessions might provide the improvement of buffering capacity leading to a shift of RCP to higher intensities and improve high-intensity exercise tolerance, and consequently enhancing the ICB phase.

The EXG was able to continue to exercise for longer periods of time above the VT during the incremental treadmill test after training. In the EXG, the higher RER<sub>max</sub> recorded after the RST, which may have been due to the longer exercise time above the VT. Although we could not directly measure blood lactate levels, the increase in RER<sub>max</sub> may reflect a greater accumulation of lactate after the RST. It has been shown that the sprint training led to a greater accumulation of lactate both in the muscle and blood during exhaustive exercise. These higher lactate concentrations after sprint training may be explained by the increase of the muscle buffer capacity and glycolytic enzymes. A large accumulation of lactate and H+ during high intensity exercise may provide an important stimulus for adaptations of the muscle pH regulating systems.

It has been shown that athletes with high VO<sub>2max</sub> have longer ICB phase than athletes with low VO<sub>2max</sub>. On the other hand, recently it has been shown that the relative ICB phase can be useful for predict both the aerobic and anaerobic capacity in the athletes. The studies that address ICB phase in relation to physical training are scarce. The increase in ICB phase with concomitant improvement in aerobic capacity after RST in the present study is consistent with the findings of Oshima at al. These authors have reported the absolute ICB phase in runners significantly increased after endurance training above the AT. They observed that the increase in the RCP is larger than that of VT after 6-month training period. In contrast, Chicharro at al. have reported that both the absolute and relative ICB phase and VO<sub>2max</sub> in professional cyclists remained unchanged throughout the season despite a considerable increase in training loads. Several studies have shown that the longer relative ICB phase in sprint-trained athletes than in endurance athletes. According to Chicharro at al., intense training sessions involving anaerobic metabolism improves the buffering capacity leading to a shift in RCP towards higher intensities, and consequently extending the ICB phase. On the other hand, training sessions involving aerobic metabolism may induces a similar shift in both VT and RCP. Therefore, it appears that effect of the high intensity training sessions RC may greater than AT. During the ICB phase, more H+ has been reported buffered by the non-bicarbonate buffer system in sprint-trained cyclists than in endurance cyclists. Sprint training has been reported to increase the muscle buffer capacity, whereas endurance training had no effect. We used a training program based on RST. Likely, the incomplete recoveries which characterize RST contribute to the aerobic adaptation of the muscle. It is, therefore, possible that, the increase in ICB phase noted in this study could be the result of the enhanced both bicarbonate and non-bicarbonate buffering capacity.

The HHV phase reduced significantly in the EXG after RST when expressed as VO<sub>2</sub>, but not as running speed. Chicharro at al. have shown that the increase in RCP leading to a shortening of the HHV phase over the season whereas remained unchanged in VO<sub>2max</sub>. In contrast, Oshima at al. have been reported that no significant change in the HHV phase after training, whereas improved in ICB phase, RCP and VO<sub>2max</sub>. Many studies have shown that the HHV phase is not correlated with VO<sub>2max</sub>. Thus it seems likely that the HHV phase is not related to exercise capacity.

CONCLUSION

From these results, we conclude that repeated sprint training can enhance the ICB phase of volleyball players which may be attributable to improve of the buffering capacity leading to shift of RCP to higher intensities without any change in VT. The increase in the ICB phase may an important factor in relation to the improvement in the high-intensity exercise tolerance in athletes.

All authors declare no potential conflict of interest related to this article.

AUTHORS CONTRIBUTIONS: Each author made significant individual contributions to this manuscript. SKE (0000-0002-3680-3580)* and KK (0000-0002-5608-2661)* were responsible for data collection, data analysis and interpretation, and the writing of the draft; HP (0000-0001-7299-0531)* helped in data analysis and interpretation; SA (0000-0003-3230-5864)* designed the study and supervised the data collection, analysis, and supervised the writing of the manuscript. All authors read and approved the final manuscript. *ORCID (Open Researcher and Contributor ID).
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