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Franck Brocherie^{1,2}, Grégoire P. Millet^{1,2}, Anna Hauser^{1,2,3}, Thomas Steiner³, Julien Rysman⁴, Jon P. Wehrlin³, and Olivier Girard^{1,2,5}

¹ISSUL, Institute of Sports Sciences, University of Lausanne, Lausanne, Switzerland; ²Department of Physiology, Faculty of Biology and Medicine, University of Lausanne, Lausanne, Switzerland; ³Swiss Federal Institute of Sport, Section for Elite Sport, Magglingen, Switzerland; ⁴Faculty of Motor Sciences, Université Libre de Bruxelles, Brussels, Belgium; ⁵ASPETAR, Qatar Orthopaedic and Sports Medicine Hospital, Athlete Health and Performance Research Centre, Doha, Qatar

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Franck Brocherie^{1,2}, Grégoire P. Millet^{1,2}, Anna Hauser^{1,2,3}, Thomas Steiner³, Julien Rysman⁴,

Jon P. Wehrlin³, and Olivier Girard^{1,2,5}

¹ISSUL, Institute of Sports Sciences, University of Lausanne, Lausanne, Switzerland;

²Department of Physiology, Faculty of Biology and Medicine, University of Lausanne,

Lausanne, Switzerland; ³Swiss Federal Institute of Sport, Section for Elite Sport,

Magglingen, Switzerland; ⁴Faculty of Motor Sciences, Université Libre de Bruxelles,

Brussels, Belgium; ⁵ASPETAR, Qatar Orthopaedic and Sports Medicine Hospital, Athlete

Health and Performance Research Centre, Doha, Qatar

Corresponding author:

Franck Brocherie

ISSUL, Building Geopolis, Campus Dorigny, University of Lausanne, CH-1015, Lausanne,

Switzerland.

Ph. +41 21 692 32 94

Fax. +41 21 692 32 93

Email franck.brocherie@unil.ch

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Running title: "Live High-Train Low and High" in team sports

ABSTRACT

Purpose: To investigate physical performance and hematological changes in 32 elite male teamsport players after 14 days of 'live high-train low' (LHTL) in normobaric hypoxia (≥14 h.day⁻¹ at 2800-3000 m) combined with repeated-sprint training (6 sessions of 4 sets of 5 x 5-s sprints with 25 s of passive recovery) either in normobaric hypoxia at 3000 m (LHTL+RSH, namely LHTLH; n = 11) or in normoxia (LHTL+RSN, namely LHTL; n = 12) compared to controlled 'live low-train low' (LLTL; n = 9). **Methods:** Prior to (Pre-), immediately (Post-1) and 3 weeks (Post-2) after the intervention, hemoglobin mass (Hb_{mass}) was measured in duplicate (optimized carbon monoxide rebreathing method) and vertical jump, repeated-sprint (8 x 20 m - 20 s recovery) and Yo-Yo Intermittent Recovery level 2 (YYIR2) performances were tested. **Results:** Both hypoxic groups increased similarly Hb_{mass} at Post-1 and Post-2 in reference to Pre-(LHTLH: +4.0%, P<0.001 and +2.7%, P<0.01; LHTL: +3.0% and +3.0%, both P<0.001), while no change occurred in LLTL. Compared to Pre-, YYIR2 performance increased by ~21% at Post-1 (P<0.01) and by ~45% at Post-2 (P<0.001) with no difference between the two intervention groups (vs. no change in LLTL). From Pre- to Post-1 cumulated sprint time decreased in LHTLH (-3.6%, P<0.001) and in LHTL (-1.9%, P<0.01), but not in LLTL (-0.7%), and remained significantly reduced at Post-2 (-3.5% P<0.001) in LHTLH only. Vertical jump performance did not change. Conclusion: 'Live high-train low and high' hypoxic training interspersed with repeated sprints in hypoxia for 14 days (in-season) increases Hb_{mass}, YYIR2 performance and repeated-sprint ability of elite field team-sport players with the benefits lasting for at least three weeks post-intervention. Key words: HEMOGLOBIN MASS, HYPOXIA, REPEATED-SPRINT ABILITY, TEAM SPORTS

INTRODUCTION

Until now, altitude training has mainly been used by individual 'endurance' athletes with the primary goal of further improving exercise performance upon return at sea level. To reach this goal, several paradigms - 'live high-train high' (residing and training at altitude) and more recently 'live high-train low' (sleeping at altitude but training near sea level; LHTL) approaches (30, 31) - have subsequently been introduced. To date, and despite on-going debate on its efficiency with elite endurance athletes or the nature of its underlying mechanisms (17, 24), LHTL is widely recognized as the 'Gold-standard' altitude training method (23) for athletic performance enhancement. Its success belongs to an erythropoietic effect of chronic hypoxia initiated by continuous residing at natural/terrestrial (hypobaric hypoxia) or simulated (normobaric hypoxia) altitude, whilst the possibility to maintain high training intensity and rates of oxygen flux at sea level (43).

An emerging concept is that positive gains associated with the LHTL method may rely on the magnitude of hypoxia-induced increase in hemoglobin mass (Hb_{mass}) (24). Reportedly, LHTL was found less efficient in (endurance) athletes with high pre-intervention Hb_{mass} values (16). Since team-sport (28, 41) athletes are generally characterized by moderate Hb_{mass} (24) and/or maximal oxygen uptake (VO₂max) values, one may speculate on substantial gains in Hb_{mass} following LHTL. Since aerobic metabolism dominates the energy delivery in most team sports (e.g. soccer, rugby or field hockey), it is likely that LHTL would benefit some team-sport athletes (13). Although positive short-term benefits on Hb_{mass} have been reported after only 13-19 days of exposure in Australian football league (AFL) (28), soccer (41) or Water-polo (12)

players, the level of evidence of the usefulness of LHTL to improve sport-specific performance in team-sport athletes is still limited (2).

Team sports share the common features of high-intensity, intermittent exercise patterns where the ability of repeatedly perform near or maximal bouts with incomplete recoveries (i.e., repeated sprint ability, RSA) for sustained periods is important for match outcome. In field hockey, for instance, although the distance covered by high-intensity running (~8%) and sprinting (~1%) only represent a small percentage of the total match duration, both decrease from the first to the second half, which may increase chances to evade an opponent and create scoring opportunities (26). In order to better resist fatigue in the most intense periods of a game or towards match-end, innovative hypoxic training methods have recently been tailored for team sports use (2, 13, 29). In this vein, we have recently updated the panorama of the different hypoxic methods currently available to add the repeated sprint training in hypoxia (RSH) (30) as a new form of 'live lowtrain high' regime. Briefly, RSH, which includes maximal-intensity efforts under moderate hypoxic conditions, has proved superior to similar training in normoxia (RSN) at enhancing peripheral adaptations (9, 33, 38) and thereby RSA (8, 9, 11). With physical performance only acutely (with days) assessed after the RSH intervention, however, the long-term (few weeks) deacclimatization effects (if any) of this hypoxic method are currently unknown. While all available RSH studies so far have been conducted in a laboratory environment only two have adopted a running mode (i.e., non-motorized treadmill; (5, 11)), yet none of them have used over-ground sprints, which would considerably increase the ecological validity of the literature findings.

Evaluating the combination of altitude training methods and the effect that this may have on the magnitude and time course of several aspects of match-related performance and adaptive physiological response is an integral part of the role of the research scientist. The capacity of team-sport athletes to repeatedly perform high-intensity actions not only depends on their Hb_{mass} but also on skeletal muscle tissue adaptations and the efficiency of their neuromuscular system. Theoretically, for team-sport athletes and coaches looking to elicit concurrent aerobic and anaerobic adaptations to improve sea-level performance, 'live high-train low and high' (the so-called LHTLH; *i.e.*, 2-3 weeks sleeping at 2500-3000 m with training at sea level except for few (2-3) hypoxic training sessions per week), as suggested as early as 2010 (30, 31), is an attractive combination. However, it is currently unknown whether combining LHTL and RSH in a cohort of teams-sport athletes would produce larger performance gains than concurrent LHTL and RSN.

Using a randomized, double-blinded, controlled design, the aim of this study was therefore to investigate the immediate (few days) and prolonged (3 weeks) effects of the 'traditional' LHTL approach combined with either RSH or RSN (both compared to a control) on team-sport specific sea-level performance and Hb_{mass} in elite field hockey players. We hypothesized that, combined to a traditional 'live high-train low' exposure, repeated sprint training in hypoxia (LHTL+RSH; namely LHTLH) *versus* normoxia (LHTL+RSN; namely LHTL) provides similar hematological adaptations but larger sport-specific physical performance gains, persisting at least 3 weeks post-intervention.

METHODS

Subjects. Thirty-six lowland elite male field hockey players (age 25.3 ± 4.6 years, height 178.4 \pm 6.0 cm, body weight 75.8 \pm 7.9 kg and estimated VO₂max 52.1 \pm 1.9 mL.min⁻¹.kg⁻¹ (22)) were recruited among Belgium, Spanish and Dutch first division clubs (9 of the participants were national team members of their respective countries) to participate in this study. The experiment was approved by the Anti-Doping Lab Qatar institutional review board (Agreement SCH-ADL-070) and conformed to the current Declaration of Helsinki guidelines. Subjects gave their written, informed consent after they were informed in detail of all experimental procedures and possible risks (e.g. severe intensity nature of the proposed exercise, acute mountain sickness (AMS) including headache, dizziness, tiredness, shortness of breath and nausea in isolation or in combination) associated with the experiments. Exclusion criteria for participation were acclimatization or exposure to hypoxia of more than 2000 m for more than 48 h during a period of 6 months before the study, and any history of altitude-related sickness and health risk that could compromise the subject's safety during training and/or hypoxia exposure. During the study, one subject from the control group (see next section) was excluded after the lead-in period due to insufficient fitness level (i.e., incapacity to satisfy the criterion score for physical performance tests; see testing section), while 3 others (control group: n = 1; experimental groups: n = 2) were excluded due to illness or injury.

Study design

The experimental design (Fig. 1) consisted of two weeks lead-in period (from mid-December to beginning-January) at sea level where training sessions were supervised and load quantified; a week pre-intervention period at sea level where baseline testing (Pre-) was performed; a 14-d hypoxic intervention period; and finally, a 3-wk post-intervention period at sea level with training sessions supervised and load quantified where Post-1 (2-3 days) and Post-2 (22-23 days) test sessions were performed.

Each of the three test sessions (Pre-, Post-1 and Post-2) was 48 h in duration and involved the 32 players on the same sea level testing site (Belgium). At this occasion, physical performance and Hb_{mass} were evaluated in an invariant order under similar temperate conditions (±2 h). Following the completion of Pre-, subjects were randomly assigned to one of the three following groups according to their initial fitness level and playing position (Table 1): 14 days of 'live high-train low' altitude training camp (LHTL; > 14h.day⁻¹ and simulated altitude of 2500-3000 m) during which players trained (*i.e.*, regular field hockey practice) at sea level with the addition of six repeated sprint training sessions either in normobaric hypoxia simulating an altitude of 3000 m (LHTL+RSH, namely LHTLH; n = 11) or normoxia (LHTL+RSN, namely LHTL; n = 12) and a live low-train low' group (LLTL, n = 9). LLTL players resided at sea level, yet under similar comfort conditions than the two experimental groups. While LLTL players were not enrolled in the training camp (Qatar – January 2014, normal environmental conditions) they followed the same training/competition routine (*i.e.*, without the completion of any additional repeated sprints training session) than LHTLH or LHTL. All subjects were familiar with testing procedures as

part of their regular physical performance assessment implemented in their clubs. Although not recorded, a particular attention was paid to food intake, hydration and sleep habits during the experiment so that players from all three groups were provided with similar diets and bedtime schedule, which were based on club's guidelines and the experience gained from previous training camps.

To evaluate physical performance, subjects performed a test battery at sea level on a well-ventilated indoor synthetic ground (Taraflex®) gymnasium at a constant temperature of ~22°C. Pre-, Post-1 and Post-2 testing sessions were performed in the exact same sequence as follows: (i) jump tests, after 10 min of rest, (ii) repeated sprints, and after an additional 15 min of recovery, (iii) Yo-Yo Intermittent Recovery test level 2 (YYIR2). Due to the extreme intensity of the tests, subjects were asked to arrive at the testing sessions in a rested and hydrated state, at least 3 h postprandial and having avoided strenuous training in the preceding 24 h. In all cases, subjects were asked to reproduce their last meals avoiding alcohol and caffeine intakes during the 24 h before each test scheduled in the same time slot. Tap water was provided ad libitum. For all tests, subjects were vigorously encouraged during all efforts. Prior to the testing battery, a standardized 15 min warm-up including athletic and acceleration drills was supervised by two investigators.

Living hypoxic exposure. The sleeping and recreational hypoxic facilities were fully furnished normobaric hypoxic rooms with O_2 filtration (CAT system, Colorado Altitude Training, Louisville, Colorado, USA). Three days prior to the study start, all rooms were controlled and

calibrated by qualified engineers. Furthermore, all investigators, except the main investigator, were blinded toward the group assignment. In all rooms, the air pumps were constantly turned on. O₂ fraction in each room was continuously monitored from independent O₂ probes connected to a control panel located in a room with restricted access to the main investigator only. The two intervention groups were exposed to a normobaric hypoxia equivalent to 2500 m (F_iO₂ 15.1%, BP 768.0 mmHg, P_iO₂ 108.3 mmHg) for the first 24h of the intervention period (day 1). Thereafter, the O_2 fraction was further decreased to the equivalent of 2800 m (F_iO_2 14.5 \pm 0.1%, BP 766.8 \pm 1.1 mmHg, P_iO_2 104.5 \pm 0.6 mmHg; days 2-5) and 3000 m (F_iO_2 14.2 \pm 0.1%, BP 765.3 ± 1.5 mmHg, P_iO_2 101.7 \pm 0.8 mmHg; days 6-14). Subjects were strictly confined (as verified by the main investigator) to their rooms from 22:00 to 07:00, from 08:00 to 10:00 and again from 13:00 to 16:00 during these 2 weeks. However, they were encouraged to spend more time in their rooms if desired. Concentrations of ferritin (143.6 \pm 68.9 µg.L⁻¹, range: 45-279 $\mu g.L^{-1}$) and soluble transferrin receptor (254.7 \pm 33.3 mg.dL⁻¹, range: 202-330 mg.dL⁻¹) measured during the lead-in period indicated that none of our subjects was iron deficient at the time of entering the study.

Daily physiological measures and questionnaires. During the intervention period, arterial oxyhemoglobin saturation (SpO₂) and heart rate (HR) were recorded in a blind manner using fingertip pulse oximeters (GO₂TM Achieve 9570-A, Nonin, Plymouth, MN, USA) every morning upon waking up. Afterwards, participants had to fill three different questionnaires. First, the Lake Louise score questionnaire that included five simple questions (0-3 scale) sensitive in quantifying AMS severity (34). The overall Lake Louise score was determined by summing all scores. Second, the Daily Analysis of Life Demands for Athletes (DALDA) questionnaire (36),

which is used to monitor psychological status (*i.e.*, mood state). Parts A and B of the DALDA represent the sources and manifestation of stress (general fatigue and feelings) in the form of signs and symptoms, respectively. For both parts, the number of items marked as 'worse than normal' (*i.e.*, 'a' scores) was tallied and reported. Third, the 15-item Groningen Sleep Quality Scale (GSQS) was used to evaluate high altitude sleep disturbance (42). Finally, subjects were weighted in minimal clothing with a digital balance (±0.1 kg, Seca, Hamburg, Germany) before breakfast. All aforementioned variables were averaged over the 14-d training camp for subsequent analysis.

Field hockey training sessions. During the entire study (from the start of the lead-in period to Post-2) each field hockey training session and match was monitored. Players' training loads (arbitrary units, a.u.) were calculated as total training/competition duration (min) \times session rating of perceived exertion (RPE, 6-20 Borg's scale), collected within 10 min of completing each training session. On days with two training sessions, the daily training load was taken as the sum of the sessions performed. On average, tested players practiced \sim 7.0–9.0 h.week⁻¹ (3-4 field hockey sessions + 2-3 fitness sessions + 1-2 matches) during the season (*i.e.*, within the 3 months preceding the lead-in period).

Supervised training protocol. In addition to their usual field hockey practice, players of the two intervention groups completed six specific repeated-sprint training sessions during the 14-d intervention period with at least 36 h recovery between each one. Sessions were completed on a synthetic grass ground, inside a mobile inflatable simulated hypoxic equipment (Altitude

Technology Solutions Pty Ltd, Brisbane, Queensland, Australia) as recently described (14). Briefly, it comprised a polyvinyl chloride inflatable running lane tunnel (length = 45 m, width = 1.8 m and height = 2.5 m) and a state-of-the-art hypoxic trailer (55 kW screw compressor), generating over 3000 Lpm of hypoxic air with F_iO_2 between 21% and 10% (a simulated altitude up to 5100 m). F_iO_2 was continuously measured (every 5 s) by two sensors located at 15 and 30 m in the tunnel, and displayed on the panel control, managed only by the main investigator. Air input flow was sufficient for safe, comfortable and stable training conditions with temperature and humidity maintained at ~25 °C and ~55% relative humidity. For RSH, ambient air was mixed with nitrogen (from pressurized tanks) to reduce inspired oxygen fraction (F_iO_2) to ~14.5% in order to simulate an altitude of 3000 m. In order to blind subjects to altitude, the system was also run for RSN with a normoxic airflow (F_iO_2 21.0%) into the tunnel. For motivational reasons and to reinforce the subjects' blinding regarding group classification, all players were assigned to different teammates during each of the six training sessions.

Specific training sessions. Each session lasted ~50 min including a 15 min warm-up, the repeated-sprint training routine and a 10 min recovery phase (*i.e.*, a total of 300 min for the 6 sessions among the 14-d training camp). Specifically, the repeated-sprint training routine included 4 sets of 5 x 5-s maximal sprints interspersed with 25 s of recovery with 5 min of passive recovery between sets, finally ending by a 10-min cooling-down period (Fig. 1B). Subjects were constantly reminded to perform 'all-out' efforts trying to reach peak acceleration and maintain the highest possible running speed for every 5-s sprint bout. Up to six subjects trained simultaneously in the inflatable marquee. Commercially available energy drinks and bottled water was provided ad libitum during training to ensure appropriate hydration.

Blinding. This research was run in a double-blinded, controlled manner. With the exception of the control group, subjects of both the LHTLH and LHTL groups were told (based on the head coach's request to increase team motivation) that they were both residing and training in hypoxic conditions, yet without any accurate information about the simulated altitude levels inside the rooms and inflatable marquee. The efficacy of the blinding process was evaluated upon experiment termination (*i.e.*, immediately after Post-2) by administering Lickert scales (100 m marks from 0 to 4000 m), where each participant had to indicate (separately) at which simulated altitude he believed to be living and training for the first and the second week of the altitude camp (*i.e.*, scores for week 1 and 2 were then averaged so as to report only one value for the total duration of the camp).

Pre-, Post-1 and Post-2 testing sessions

Vertical jumps. Players performed the following vertical jump tests with the hands kept on the hips to eliminate any influence of arm swing: (i) squat jump (SJ) starting from a static semi-squatting position (\sim 90° of flexion) maintained for \sim 1 s and without any preliminary movement, (ii) countermovement jump (CMJ) starting from a standing position, squatting down to \sim 90° angle and then extending the knee in one continuous movement and (iii) one set of multi-rebound jumps (MRJ) with rebounds to the highest possible point six times. For SJ and CMJ, subjects were asked to perform two maximal trials and the highest jump was recorded. During MRJ, they were instructed to keep their knees as stiff as possible ('ankle jumps') and to have as brief a contact time as possible. Jump heights were calculated by recording the flight times (SJ, CMJ and MRJ) and ground contact (MRJ) with an optical measuring apparatus (Optojump, Microgate, Bolzano, Italy).

Repeated sprints. The subjects underwent a RSA test consisting of eight 20 m sprints departing every 20 s. The sprints were performed in a back and forth format to allow for passive recovery during the short rest period. Players had to complete the distance in a straight line as fast as possible. Three seconds prior to the start of each sprint, they were asked to assume the ready position and await the start signal with a 3-s countdown (3-2-1-'Go'). Each sprint was initiated from a standing position, 50 cm behind the photocell gate, which started a digital timer. Sprint times were measured to the nearest 0.01 s using photocells connected to an electronic timer (Polifemo Radio Light, Microgate, Bolzano, Italy), which height was adjusted according to the height of the subject's hip. The two photocells were placed at 0 and 20 m distance intervals. During the first sprint, subjects were required to achieve at least 95% of their criterion score (i.e., defined from the best of three single 20-m sprints interspersed with 2 min recovery – data not presented), as a check on pacing. All of the subjects satisfied this criterion score. Two scores were calculated during the RSA test: the cumulated sprint time and the percent sprint decrement calculated as follows: [(cumulated sprint time) / (best sprint time \times 8) – 1] \times 100 (15). A similar RSA test (i.e., 6 x 30 m departing on 25 s) in highly-trained field hockey players was found very reliable, as evidenced by a typical error of 0.7% for the total sprint time (39).

Specific aerobic capacity. To assess high-intensity intermittent running performance, subjects performed an incremental running test to exhaustion (YYIR2) (1). Briefly, the test consisted in repeated 20-m shuttle runs at increasing speeds (starting at 13 km.h⁻¹) controlled by audio beeps interspersed by 10 s of active recovery. When the subject failed to reach the finishing line in time twice, the distance covered was then recorded and represented the test result. Heart rate (Polar Electro, Kempele, Finland; 5 s average) was measured with the highest value retained as

maximal heart rate (HR_{max}). During the YYIR2 test, none of the subjects reported a HR_{max} <95% of their age-predicted HR_{max} (i.e. traditional 220-age formula) indicating maximal exhaustion. This test is reproducible and a sensitive tool for assessing aerobic capacity in team-sport players (20).

Hemoglobin mass. Hb_{mass} was measured in duplicate at each time point by using a slightly modified version (40) of the optimized carbon monoxide (CO)-rebreathing method described by Schmidt and Prommer (37). Briefly, subjects spent 5 min in a sitting position before three capillary blood samples (35 µl) were taken from the earlobe and analyzed immediately for the baseline carboxyhemoglobin values (ABL 800flex, Radiometer A/S, Copenhagen, Denmark). Subjects then rebreathed for 2 min a gas mixture of 100 mL pure CO (Multigas SA, Domdidier, Switzerland) and 3.5 L oxygen in a closed circuit system (glass spirometer, Blood Tec GbR, Bayreuth, Germany). During the rebreathing period a CO gas analyzer (Dräger PAC 7000, Dräger Safety, Lübeck, Germany) was used to check for possible CO leakage at the nose, mouthpiece and spirometer system. At minute 6 and 8 after starting CO rebreathing, two final capillary blood samples were taken from the earlobe and averaged as a 7 min post carboxyhemoglobin value. Directly before and 2 min after the rebreathing, the same CO-gas detector as described above was used to quantify the end-tidal CO concentration in parts per million. Hb_{mass} was calculated from the mean change in carboxyhemoglobin before and after CO rebreathing, as described previously by Steiner and Wehrlin (40). Both measurements were performed on two consecutive days (12- to 24-h time lag between the measures) and the results were averaged. In our mobile laboratory, the typical error was 1.6% for CO-rebreathing method and 1.1% for averaged duplicate Hb_{mass} measurement over all measurement time points.

Statistical analysis

All data in text and figures are presented as mean \pm SD. Relative changes (%) in performance are expressed with 95% confidence interval (95% CI). LHTLH and LHTL room and inflatable marquee exposures were compared with a paired t-test. One-way ANOVA was used to test differences in training load, questionnaires and physiological measures between groups. Two-way ANOVA with repeated-measures [Time (Pre- vs. Post-1 vs. Post-2) \times Condition (LHTLH vs. LHTL vs. LLTL)] was used to compare physical performance and Hb_{mass} data. ANOVA assumptions were verified preceding all statistical analyses; Pairwise differences were identified using the Holm-Sidak post-hoc analysis procedure adjusted for multiple comparisons. Pearson's product-moment correlation analysis was employed to determine the correlations between Pre- and Post-1 and/or Post-2 changes between Hb_{mass} and physical performance tests. Null hypothesis was rejected at P < 0.05. All statistical calculations were made using Sigmaplot 11.0 software (Systat Software, San Jose, CA).

RESULTS

Hypoxic dose and efficacy of the blinding procedure. The average daily room confinement $(14.5 \pm 0.8 \text{ and } 14.4 \pm 0.7 \text{ h.day}^{-1}, P = 0.52)$, the total RSH/RSN exposure $(3.9 \pm 0.7 \text{ and } 3.7 \pm 1.1 \text{ h}, P = 0.51)$ and the total $(202.5 \pm 4.5 \text{ and } 201.2 \pm 3.0 \text{ h}, P = 0.75)$ hypoxic dose were similar between LHTLH and LHTL. Participants from both experimental groups were not able to correctly identify the simulated altitude where they were residing at [average: $2591 \pm 767 \text{ m}$ (range: 200-3700 m) and $2491 \pm 658 \text{ m}$ (range: 1500-3500 m) for LHTLH and LHTL, respectively] and training in [average: $2445 \pm 771 \text{ m}$ (range: 1000-4000 m) and $2648 \pm 761 \text{ m}$

(range: 1000-3750 m) for LHTLH and LHTL, respectively]. Overall, this indicates that the blinding process was successful, and subjects were unaware of the hypoxic group classification.

Morning heart rate, SpO₂ and questionnaires

No difference (P = 0.74) in average values of wake-up HR was found between groups during the experimental period (60 ± 8 , 60 ± 3 and 59 ± 7 bpm for LHTLH, LHTL and LLTL, respectively). Mean SpO₂ for LLTL ($97.2 \pm 0.7\%$) was higher (P < 0.001) than both intervention groups and LHTLH showed lower SpO₂ than LHTL during the hypoxic exposition ($92.3 \pm 0.9\%$ and $93.2 \pm 0.9\%$, respectively; P < 0.05). No change in average body weight was observed between groups (P = 0.87) during the study.

The mean Lake Louise Score was 1.0 ± 0.9 for LHTLH, 1.2 ± 0.8 for LHTL and 1.4 ± 0.7 for LLTL and no difference (P = 0.54) was found between groups. The parts A (0.6 ± 0.5, 0.7 ± 0.8 and 0.9 ± 0.7 for LHTLH, LHTL and LLTL, respectively; P = 0.41) and B (1.2 ± 1.3, 2.1 ± 1.7 and 2.6 ± 2.1 for LHTLH, LHTL and LLTL, respectively; P = 0.14) of the DALDA were not different between groups. The average GSQSS value for sleep quality during the intervention period was comparable for all groups (2.0 ± 0.9, 2.0 ± 1.1 and 2.1 ± 1.0 for LHTLH, LHTL and LLTL, respectively; P = 0.93), indicating no disturbed sleep. Similarly, no difference was observable regarding GSQSS waking state (1.2 ± 0.6, 1.2 ± 0.6 and 1.1 ± 0.5 for LHTLH, LHTL and LLTL, respectively; P = 0.90).

Training load. Overall training load was closely matched among the three groups during the study (3534 ± 412 , 3702 ± 570 and 3179 ± 309 a.u. for LHTLH, LHTL and LLTL, respectively, P = 0.35). No difference in averaged field hockey training load occurred during the lead-in (981 \pm 142, 1054 ± 196 and 1070 ± 55 a.u., P = 0.57), 2-wk intervention (976 ± 112 , 985 ± 143 and 1016 ± 99 a.u., P = 0.86) and 3 weeks post-intervention (987 ± 142 , 981 ± 142 and 1094 ± 254 a.u., P = 0.59) periods between LHTLH, LHTL and LLTL groups. Noteworthy, no significant difference (P = 0.90) was observable in the averaged training load monitored during both specific RSH (590 ± 76 a.u.) and RSN (594 ± 35 a.u.) sessions.

Hemoglobin mass. Compared to Pre-, both hypoxic groups similarly increased their Hb_{mass} at Post-1 with no further change at Post-2 (LHTLH: 888 ± 107 , 924 ± 114 (P < 0.001) and 912 ± 127 g (P < 0.01); LHTL: 931 ± 131 , 957 ± 140 and 956 ± 137 g (both P < 0.001; Fig. 2). Noteworthy, the increase in Hb_{mass} at Post-1 and Post-2, which exceeded the typical error of 1.6% for the CO-rebreathing procedure, occurred in 18 and 15 out of the 23 subjects composing the two intervention groups, respectively. Hb_{mass} remained unchanged for LLTL: 929 ± 171 , 934 ± 170 and 930 ± 163 g at Pre-, Post-1 and Post-2, respectively. Finally, no significant correlation between Pre-, Post-1 and/or Post-2 changes in Hb_{mass} and any physical performance data could be evidenced.

Physical performance. With the exception of CMJ height (time effect, P < 0.05), none of the vertical jump test data displayed a main effect of condition or any significant interaction between time and condition (Table 2).

From Pre- to Post-1, the intervention resulted in similar increases in YYIR2 performance in LHTLH (from 520 ± 165 to 615 ± 162 m; +21% (95% CI: 7; 36%), P < 0.01) and LHTL (from 540 ± 126 to 647 ± 147 m; +22% (10; 34%), P < 0.001), whereas no significant change occurred in LLTL (from 413 ± 89 to 427 ± 96 m; +4% (-19; 26%)) (Fig. 3). At Post-2, both hypoxic groups further increased their YYIR2 performance by an average of +45% (21; 74%) (764 ± 227 m) and +19% (21; 75%) (789 ± 187 m) in reference to Pre- and Post-1 (both P < 0.001), respectively.

During the RSA test (Fig. 4), cumulated sprint time decreased from Pre- to Post-1 in both LHTLH (from 27.23 ± 1.15 to 23.23 ± 1.02 s; -3.6% (-5; -2%), P < 0.001) and LHTL (from 27.05 ± 0.81 to 26.54 ± 0.77 s; -1.9% (-3; -1%), P < 0.01) groups, with no significant change in LLTL (from 26.98 ± 1.03 to 26.81 ± 1.47 s; -0.7% (-3; 1%)). Compared to Pre-, cumulated sprint time at Post-2 remained significantly shorter (P <0.001) for LHTLH (26.21 ± 1.09 s; -3.5% (-5; -2%)), while no difference was observed for LHTL (26.63 ± 0.90 s; -1.5% (-3; 0%)) and LLTL (26.86 ± 1.31 s; -0.8% (-3; 1%)). Sprint decrement score (averaged of all conditions: 4.0% (-1; 9%)) did not change throughout the protocol (P = 0.14).

DISCUSSION

To the best of our knowledge, the present study is the first randomized, double-blinded, controlled investigation verifying the usefulness of combining hypoxic training methods when attempting to improve sea level performance in elite team-sport athletes. We have administered 'traditional' LHTL with RSH, namely 'live high-train low and high' hypoxic training (LHTLH), and compared its immediate (few days) and prolonged (few weeks) effects with a combination LHTL and RSN. Our results are clear and compelling: first, similar increases in Hb_{mass} and specific aerobic fitness (YYIR2 performance) in the two intervention groups despite a low altitude dose (≥ 200 h). Second, the YYIR2 performance was further enlarged at Post-2 in reference to Post-1 in the two intervention groups, while Hb_{mass} was maintained. Third, repeatedsprint ability was improved in the two intervention groups, yet with twice larger gains measured at Post-1 in LHTLH compared with LHTL. Three weeks after the intervention, RSA performance improvements were only maintained in the LHTLH group. Overall, this short-term LHTLH method (i.e., 14 days of LHTL exposure + 6 RSH sessions, as performed 'in-season') demonstrated a greater effect on Hb_{mass} and sport-specific physical performance (YYIR2 and RSA) over LHTL and LLTL training in elite field hockey players, with the benefits lasting for at least 3 weeks post-intervention.

LHTL(H) as a stimulus for increasing Hb_{mass}

In the present study, we have demonstrated that 14 days of LHTL in normobaric hypoxia at \sim 2800-3000 m were sufficient to immediately increase Hb_{mass} by \sim 3-4%. This increase is greater than the magnitude observed in previous studies with similar hypoxic dose in normobaric

hypoxia or is in line with longer exposure (i.e., 1%/100 h) in hypobaric hypoxia (18). More specifically, our results are very similar to those involving team-sport populations. Reportedly, Hb_{mass} increased by ~3-4% after (i) 18-19 days pre-season moderate altitude (~2100 m) training camp in elite AFL players (28), (ii) ten days of simulated LHTL in international-level water polo players (12) and (iii) after 13 days spent at altitude 3600 m in U17 soccer players (41). Direct comparisons of Hb_{mass} gains between the aforementioned studies are difficult since the magnitude of the hypoxic-induced hematological changes would differ according to various 'dose-response' relationship (25), the training content (31), and individual responsiveness (6). Of importance, with no significant difference in training load between our three groups, the lack of change in Hb_{mass} characterizing the LLTL group would indicate that any specific hockey practice/training-induced increase in Hb_{mass} is unlikely for this short period. Despite on-going debate surrounding the importance of hematological factors in driving adaptations induced by chronic exposure to hypoxia (18), it has been acknowledged that in elite endurance (16) as well as team-sport (28) athletes the hypoxia-induced Hb_{mass} response is inversely related to its initial level (27). In our study, the pre-intervention Hb_{mass} values (average of the three groups = 916 \pm 133 g) are in an acceptable agreement with those measured in other team-sport athletes (926 \pm 118 g, ranging 721-1023 g for AFL, soccer, field hockey and Water-polo players) (12, 19, 28, 41).

With similar room confinement time for our two intervention groups, the addition of 6 RSH sessions (~5 h at simulated altitude of 3000 m) for LHTLH had no measurable impact on Hb_{mass} increase. This demonstrates that the hypoxic dose is the main factor for Hb_{mass} increase and that RSH *per se* has no 'erythropoietic' effect. It is also known that at moderate altitude the

occurrence of negative side effects (e.g. acute mountain sickness symptoms) is very low (35). This was confirmed with the present athletes who displayed low perceptual scores to questionnaires. Taken as a whole, this fully supports the notion that a relatively short period of normobaric hypoxic LHTL or LHTLH exposure (~2 weeks and low hypoxic dose of ~200 h at simulated altitude of ~2800-3000 m) may be sufficient to increase Hb_{mass} in team-sports athletes.

Immediate effect on sea-level physical performance

The aforementioned increase in Hb_{mass} at Post-1 was also transposed into an immediate improved specific aerobic performance as evidenced by the large (~21%) and similar increase in the YYIR2 distance in the two intervention groups. Noteworthy, the absence of an increase in jumping performance following LHTL combined with repeated sprinting (hypoxic or normoxic conditions) would suggest that leg power was not modified in response to such training and was therefore probably not directly involved in the marked aerobic performance gains. Our substantial in-season increments in YYIR2 performance are in line with the findings of Galvin et al. (11) who reported that a 4-wk RSH treadmill sprints intervention induced +33% improvements in well-trained academy rugby players' intermittent running performance (*i.e.*, YYIR test level 1). Therefore, the present data confirm that within no more than 14 days of residing in simulated altitude, irrespective of the additional RSH training, there were substantial ergogenic benefits of LHTL for elite team-sport athletes when tested at sea level.

Along with YYIR2 performance, improvement in RSA also occurred in our two experimental groups, with a 2-fold superior benefit seen in LHTLH compared to LHTL (cumulated sprint

time: -3.6 versus -1.9%, respectively). In addition to this shorter cumulated sprint time, the unchanged percent sprint decrement observed at Post-1 strengthens this result. By using sportspecific ecological training and testing setting, i.e., repeated sprinting on synthetic grass with players wearing their field hockey shoes inside a mobile inflatable hypoxic marquee producing normobaric hypoxic conditions, the present results therefore strengthens the validity of previous studies (5, 9, 11, 33). In particular, it appears of practical relevance to solve some of the problems related to the congested calendars of the majority of professional team-sport athletes, which do not allow players to afford the time for the usually recommended 3-4 week blocks of altitude training. Furthermore it suggests that the proposed mechanisms (see below) were not blunted by the Hb_{mass} increase. The rationale for this study was based on the assumption that, if LHTL paradigm works to improve sea-level 'endurance' performance, and if additional RSH works to improve sea-level specific performance (i.e. higher tolerance for repeated-sprint exercises (5, 9, 30), then the physiological benefits of LHTLH method must derive from the combination of these two hypoxic methods. Whereas the mechanisms behind coupling different hypoxic methods are currently unknown, our findings provide evidence for both 'aerobic' and 'anaerobic' benefits to acutely improve sea-level team-sport physical performance.

While it is thought that the main mechanism for improved sea-level performance after 'traditional' LHTL exposure relies on an increase in red cell mass (23), other hypoxia-induced physiological adaptations are possible and may include an improved muscle buffer capacity (17). Similarly, exercise capacity during high-intensity intermittent tasks not only depends on Hb_{mass}, but also on molecular adaptations at the skeletal muscle level and the efficiency of the neuromuscular system (8, 9, 11). When used in isolation (8), RSH has proved superior to RSN in

enhancing peripheral adaptations (*i.e.*, oxidative capacity, capillary density and muscle glycolytic potential as well as increased expression of hypoxia inducible factor 1α (HIF- 1α) and downstream genes to oxygen and transport) (9, 38). Pending confirmatory research, this would suggest a hypoxia-induced increase in anaerobic glycolytic activity in the muscle and a more efficient use of fast twitch muscle fibers (8, 9). With comparable Post-1 Hb_{mass} gain between LHTLH and LHTL, the twice larger improvement in RSA in the former compared to the latter group is likely linked to the aforementioned RSH specific adaptations. Nevertheless, one limitation relates to our inability to examine the independent effects of repeated sprint training and residing in hypoxia. While the addition of a group of players living near sea level with additional RSN would have improved our test design, increasing our sample number was unrealistic in our cohort of elite players.

Delayed effects on sea-level performance

When players were re-tested 3 weeks after completion of the hypoxic intervention, LHTLH and LHTL groups displayed maintenance of Hb_{mass} values but an enlargement of YYIR2 performance at Post-2 in reference to Post-1, while only LHTLH players were able to preserve their hypoxia-induced RSA gains. Within competitive field hockey matches, research has reported a reduction in total distances players achieved at high-intensity (26). Bearing in mind that decisive events during competitive games often reliant on transient RSA (10, 32), the larger improvement in RSA performance in LHTLH compared to LHTL players is likely to give them a further competitive edge in the most intense periods of a game or towards match-end. Three main components have been suggested to primarily influence the rate of de-acclimatization or

change in performance (i.e., training responsiveness and exercise capacity) after an altitude training stimulus (7): the timing in decay in Hb_{mass}, the consequences of ventilatory acclimatization and the alterations in biomechanical and neuromuscular factors associated with force production. In the present study, the increase (~3%) in Hb_{mass} at Post-2 appears consistent with the model estimated by Gore et al. (18) susceptible to be maintained for up to 20 days post-LHTL. Considering the paucity of data describing the decay and/or normalization of haematological response after return to sea level, it is unclear if a period of re-acclimatization to sea level is necessary to obtain the full effect of the additional high-intensity altitude training, while this effect may well relate to ventilatory factors (i.e., time course of the decay of ventilatory acclimatization with return to sea level (44)). In our study, however, there was no difference in YYIR2 improvement in the post-altitude periods between LHTLH and LHTL, making it unlikely that the post-intervention improvement represents a generic "delayed" response to altitude. Conversely, it could be hypothesized that, due to the positive acclimatization response to hypoxia, the ability to train at a higher level after return to sea level may allow higher fitness level achievement (i.e., improved training responsiveness). While postintervention training load was strictly controlled and monitored for 3 weeks (i.e., similar between groups), it cannot be completely ruled out that easier subjective ratings to produce the same 'external physical output' may have resulted from the LHTLH intervention, which deserves further research attention. Finally, although speculative, another point to mention is that hypoxiainduced improvement in the active musculature neural drive (4) may have up-regulated musculoskeletal stiffness, leading to faster stride frequencies and thereby a better sea-level RSA (3). Reportedly, after 28 days of LHTL, where out of as many as 40 total training sessions, 7 training sessions classified as higher intensity were performed at lower altitudes (365 m to 1150

m), no alteration in stride length, stride frequency, ground contact or aerial time was observed in a group of 6 elite distance runners tested at common racing speeds (18-25 km.h⁻¹) (21). However, as this later study did not recruit team-sport participants or did not employ sprinting speeds, with measurements restricted to immediately post-intervention only, more research is required.

CONCLUSION

This study is the first to combine the traditional LHTL with repeated sprint training in hypoxia compared to similar training in normoxia in team-sport players and to determine its short- (few days) and long-term (3 weeks) effects on hematological parameters and sport-specific physical performance. With only a low hypoxic dose (≥ 200 h), 'live high-train low and high' (LHTLH) conducted for 2 weeks during the in-season period of elite field hockey players is an attractive intervention to elicit 'aerobic' and 'anaerobic' benefits for improving sea-level performance. Our results displayed similar immediate up-regulated Hb_{mass} and increase in specific aerobic performance in the two experimental groups. However, the superiority of the LHTLH over the LHTL method was demonstrated on the repeated-sprint ability test with twice larger acute performance gains, those being well maintained at least for 3 weeks post- the LHTLH intervention only. This advocates that non-hematological factors outside the role played by oxygen-carrying capacity are probably more robust to explain performance enhancement and/or maintenance following LHTLH. The determination of the optimal characteristics for combining hypoxic methods and the identification of the hematological, ventilatory and biomechanical mechanisms of adaptation and individual rates of decay in de-acclimatization of the newly proposed LHTLH method also require future research.

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Figures legend

Fig 1. Protocol overview. General procedure (a) and description of a typical repeated sprint training session (b). LHTLH – 'live high-train low and high'; LHTL – 'live high-train low'; LLTL – 'live low-train low'; RSH – repeated sprint in hypoxia; RSN – repeated sprint in normoxia; Pre- (or baseline), 2-wk after lead-in period; Post-1 and Post-2, 1 and 3-wk, respectively, after termination of the intervention period; Hb_{mass} – total hemoglobin mass measured via CO rebreathing and performed in duplicate; field testing - included vertical jumps, 30 m sprint with intervals at 5, 10 and 20 m, RSA and Yo-Yo intermittent recovery level 2; TL – training load; wellness – morning wellness monitoring, including the Lake Louise score questionnaire, The Daily Analysis of Life Demands for athletes (DALDA) and The Groningen Sleep assessment questionnaire; HR - heart rate, SpO₂ - arterial oxygen saturation.

Fig. 2. Mean changes in hemoglobin mass (Hb_{mass}) from baseline (Pre-) to the end of the intervention period (Post-1) and after 3 weeks (Post-2) for LHTLH, LHTL and LLTL groups. The dashed lines represent the typical error of the carbon monoxide rebreathing procedure in the present study (1.6%). T, C and I for time, condition and interaction effects. ** Significantly different from Pre-, P < 0.01; *** P < 0.001.

Fig. 3. Distance (m) covered during the Yo-Yo Intermittent Recovery test level 2 (YYIR2). Measurements were taken prior to (Pre-), immediately (Post-1) and 3 weeks (Post-2) after the intervention. T, C and I for time, condition and interaction effects. ** Significantly different from Pre-, P < 0.01; *** P < 0.001. ### Significantly different from Post-1, P < 0.001.

Fig. 4. Cumulated sprint times (A) and sprint decrement score (B) during the repeated-sprint ability (8 x 20 m - 20 s passive recovery) test. Measurements were taken prior to (Pre-), immediately (Post-1) and 3 weeks (Post-2) after the intervention. T, C and I for time, condition and interaction effects. ** Significantly different from Pre-, P < 0.01; *** P < 0.001.

Figure 1

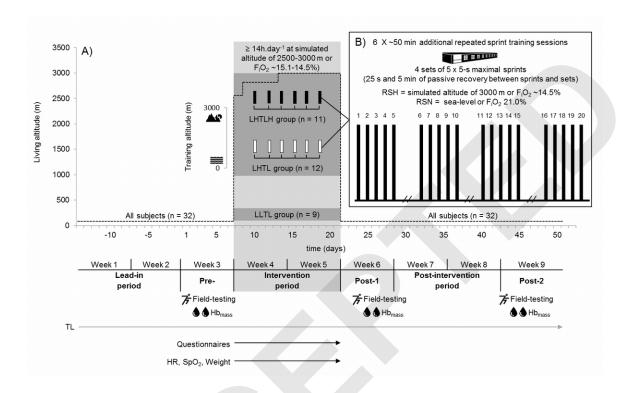


Figure 2

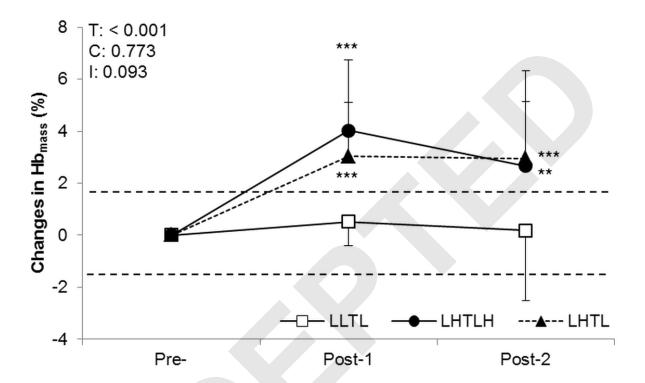


Figure 3

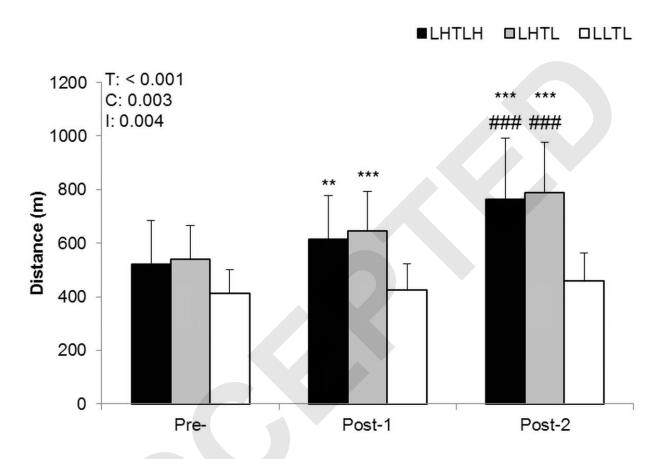


Figure 4

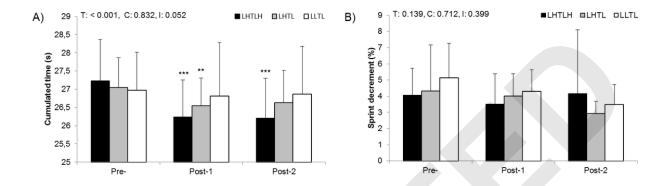


Table 1. Subject characteristics

	LHTLH	LHTL	LLTL		
Age (yr)	27.6 ± 4.8 *	25.3 ± 4.2	22.3 ± 4.6		
Height (cm)	179.1 ± 8.6	178.9 ± 4.7	178.1 ± 5.2		
Weight (kg)	76.5 ± 7.9	76.1 ± 8.6	74.3 ± 8.9		
MAV (km.h ⁻¹)	17.5 ± 0.4	17.6 ± 0.3	17.3 ± 0.2		
$RSA_{TT}(s)$	27.23 ± 1.15	27.05 ± 0.81	26.98 ± 1.03		
Position	1GK/4DF/3MF/3FW	1GK/5DF/2MF/4FW	1GK/4DF/3MF/1FW		

Values are presented as mean \pm SD for the control group (LLTL, n = 9) and the experimental groups in either hypoxia (LHTLH, n = 11) or normoxia (LHTL, n = 12).

MAV, maximal aerobic velocity; RSA_{TT} , repeated sprint cumulated sprint time, Position: GK, goalkeepers; DF, defenders; MF, midfield; FW, forward.

^{*} P < 0.05 for LHTLH older than other groups.

Table 2. Vertical jump performance results before (Pre-) and after (Post-1 and Post-2) 14-days of 'live high-train low' (LHTL) exposure and additional repeated-sprint training in hypoxia (LHTL+RSH, namely LHTLH), in normoxia (LHTL+RSN, namely LHTL) or in the control group (LLTL).

	LHTLH			LHTL		LLTL			P-value			
	Pre-	Post-1	Post-2	Pre-	Post-1	Post-2	Pre-	Post-1	Post-2	Time	Condition	Interaction
												(Time × Condition)
SJ (cm)	36.2 ± 3.2	37.3 ± 3.0	36.1 ± 3.7	36.2 ± 5.4	36.8 ± 4.8	36.3 ± 4.8	37.3 ± 5.3	38.6 ± 5.6	37.5 ± 4.5	0.080	0.757	0.903
CMJ (cm)	39.2 ± 3.5	38.7 ± 3.6	38.2 ± 3.9	38.9 ± 5.6	39.0 ± 5.5	37.3 ± 5.8	41.5 ± 5.6	41.1 ± 5.8	39.8 ± 4.5	0.013	0.506	0.924
MRJ (cm)	28.9 ± 4.4	30.9 ± 4.2	30.7 ± 4.0	30.0 ± 6.5	30.6 ± 5.6	29.8 ± 6.2	30.6 ± 5.5	32.0 ± 5.9	31.4 ± 5.7	0.128	0.847	0.895

SJ, Squat jump test; CMJ, Counter movement jump; MRJ, multi-rebound jumps.