METHODS

Experimental Design

To investigate the effects of IEH during SIE and SSG on physiological indicators and training quality in handball, we conducted a randomized, crossover, and single-blind study. Participants completed four training sessions, two SIE and two SSG, under environmental conditions of normoxia (NOR, FiO2 = 0.21, SIE-NOR and SSG-NOR) or IEH (FiO2 = 0.13, SIE-IEH and SSG-IEH).

Participants

We recruited ten handball players for this study. The characteristics of the participants are shown in Table 1, with body composition values obtained using the dual-energy X-ray absorptiometry technique (Lunar DPX-NT, General Electric Healthcare, Buckinghamshire, UK). The participants' fitness levels were classified according to criteria previously proposed by Mackay et al [1] as Tier 2: Trained/Developmental. To be included in the study, participants should be male handball players aged between 18 and 35, with no current injuries in the upper or lower limbs, while the exclusion criteria consisted of not completing one or more of the scheduled sessions (SIE-NOR, SIE-IEH, SSG-NOR, SSG-IEH). Two participants did not complete all sessions and were removed from final analysis. The participant flow chart is shown in Figure 2. We informed all participants about the risks and benefits of the study, and each provided their voluntary agreement by signing a document in accordance with the Declaration of Helsinki and approved by the university's ethics committee.

Hypoxic exposure

A balloon system previously proposed and validated by Norberto et al. [2] was used to administer hypoxia exposure during the training sessions, which allows the storage of air with low FiO_2, obtained by using a commercial hypoxia generator (ACT – 12 air unit, Everest Summit II, Cardiff, EUA). Unidirectional hoses and masks were connected to this system, allowing participants to inhale hypoxic air and exhale air into the environment, and we used a damper system which allowed the air flow to be redirected so that the participants in the normoxic situation inhaled atmospheric air. Randomization was carried out by an online drawer and only the researchers were aware of the participants' experimental conditions. After a 10-minute standardized warm-up which included mobility exercises, ballistic stretching, squats, jumps, running drills, and short sprints, the participants remained breathing the air

corresponding to the randomised condition for 5 minutes and then began their first effort. Between efforts, the recovery and exposure time was 4 minutes (4x) and after the last effort another 5 minutes of passive exposure, for a total of 26 minutes of exposure (Figure 1).

Training protocol

The first two session were SIE and the last two SSG, in a non-randomised way. We chose this format to avoid a dropout after the SSG sessions had been carried out – if this were to happen, it would be necessary to repeat the session with all the participants due to the crossover design. The sessions were carried out at least 48 hours apart. In addition, participants were instructed not to perform intense physical exercise in the 24 hours prior to the study protocol sessions.

Sprint interval exercise (SIE)

The SIE sessions consisted of five maximal running efforts lasting 30 seconds, with a 4-minute break between each effort. The efforts were made between the side lines of the court, with a 180º change of direction every 20 metres. The assessors verbally encouraged the participants to make a maximum effort throughout the training session. The sessions were filmed and then the distances covered in each of the 5 efforts were determined, with a precision of 2.5 metres (±1.8% of the average value of all the sprints). In addition to the distance values, the average power output (PO) during the effort was also estimated using the following formula:

$$\text{Eq 1: } Power\ output\ (W) = \frac{Body\ weigth\ (kg) * [distance\ (m)]^2}{[time\ (s)]^3}$$

Due to the time spent changing direction and the consequent decrease in speed and distance travelled during the 30 s, this estimated PO is underestimated. Even so, we decided to use it as a value that includes the external load that had to be moved to make the effort (body mass) and the effort itself (the distance travelled). Previous studies have already used this approach on straight line repeated sprints protocols [3]. PO was analysed as maximum (PO in the longest sprint, PO_{MAX}), mean (average of all sprints, PO_{MEAN}) and fatigue index:

$$\text{Eq 2: } Fatigue\ index(\%) = 100 - \left(\frac{PO_{MEAN}}{PO_{MAX}}\right) * 100$$

Small-sided games (SSG)

The sessions with SSG consisted of five games lasting 2.5 min with a 4 min break between each game, on an official court (40 × 20 m). Each team consisted of four court players plus a goalkeeper (who was not exposed to hypoxia at any time). Two rules were changed in order to increase the intensity of the session: dribbling was not allowed and the ball was replaced directly by the goalkeeper, even after a goal [4]. To ensure that the technical level of the

teams was balanced, a coach who knew all the players divided them into pairs of a similar level and then they were randomised within this pair. The teams were maintained for both games.

To assess performance during the SSG, the sessions were recorded and then analysed using the PlayerScore observation tool [5]. This systematic observation tool makes it possible to classify different actions in the offensive and defensive phases of the game, with positive and negative scores based on how much a given action contributes to or impedes the team in scoring a goal or preventing a goal.

Internal load

Heart rate

Heart rate values were monitored during the SSG and SIE sessions. The values were recorded immediately after the efforts and every 10 s during all the recoveries. We analysed the values as the maximum value (immediately after the effort, HR_{MAX}) during the session and the sum of the area under the curve (HR_{AUC}) [7] during recovery bouts.

Blood lactate

Before warming up and one minute after the end of each effort, 25 µL of blood was collected from the earlobe in a previously heparinised glass capillary and stored in a tube containing 50 µL of 1% NaF. The samples were frozen at -20 ºC and the lactate concentration ([La]) was estimated on a biochemical analyser calibrated according to the manufacturer's instructions (YSI 2700 Biochemistry Analyzer, Yellow Springs Instrument Company, Ohio, EUA). The [La] values were then analysed as the difference between the resting value and the maximum value obtained during the session (Δ [La]).

Perceptual responses

A modified Borg scale (0-10) was used to monitor perceptual responses. The four responses monitored were: the rate of perceived exertion (*How much effort did you give compared to a maximal effort?*), the perception of overall discomfort (*How uncomfortable do you feel overall?*), the perception of difficulty breathing (*How uncomfortable does it feel to breathe?*), and the perception of discomfort in the lower limbs (*How uncomfortable do your legs feel?*) [8].

Data analysis

The data was analysed using generalised linear mixed models, with fixed effects for training modality (SSG or SIE) and environmental condition (IEH or NOR) and random effects for individuals. For the variables relating to distance or PO in SIE or PlayerScore in SSG, the only fixed effect was the environmental condition. Before fitting the models, the presence of outliers was checked using box and scatter plots. The Gamma and Gaussian distributions were

tested, and the assumptions of normality of the residuals were checked by visually inspecting the histogram and the QQplot, as well as homoscedasticity by analysing the Pearson residual plot as a function of the predicted values. If the assumptions of the models were met, the final model adopted would be the one with the lowest Akaike information criterion (AIC). Based on the model selected, hypothesis tests were carried out on the coefficients, with Bonferroni correction, adopting a significance level of 5%.

The data is presented as estimated mean or mean difference and 95% confidence interval. Cohen's d for paired samples was used to estimate the effect size, calculated as the quotient of the difference between the means and the product of the standard error of the difference and the square root of the sample size. The qualitative interpretation of the effect size was: small (0.0-0.2), medium (0.2-0.5), large (0.5-0.8) and very large (>0.8). The analyses were conducted in the R environment [9], with the following packages: readxl [10], dplyr [11], writexl [12], lme4 [13], ggResidpanel [14], ggplot2 [15], lmerTest [16], and emmeans [17].

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