Effects of intermittent negative pressure and active recovery therapies in the post-match period in elite soccer players: A randomized, parallel arm, comparative study

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Summary

Study aim: This study compared the effects of intermittent negative pressure therapy (INPT) vs. active recovery therapy (ART) on post-match physiological parameters such as serum CK level and skin temperature of the lower limbs in elite soccer players.

Material and methods: Twenty healthy male professional soccer players from a Brazilian first division soccer club were enrolled in this randomized, parallel arm, open label, comparative study. After participating in 2 soccer matches, they were randomly assigned to two groups (n = 10) to receive a 30-min session of INPT (intermittent exchange of hypobaric pressure range 33 to 51 mmHg) or ART (self-myofascial release, mobility and stability exercises, and cycle ergometer exercise). The intervention was conducted after a match with assessments immediately before and after the intervention and again 24 h after the intervention.

Results: A significant interaction effect ($F_{2,36} = 4.503$, $p = 0.018$, $\eta^2 = 0.130$) was observed, indicating that the decrease of CK from pre-intervention to 24 h post-intervention was greater in the INPT than in the ART group. Lower limb skin temperature was significantly lower after INPT than after ART ($p < 0.003$).

Conclusions: Serum CK level and skin temperature of lower limbs showed better recovery up to 24 h after the intervention with INPT in elite soccer players.

Keywords: Creatine kinase – Thermal images – Soccer – Sports performance – Recovery

Introduction

Soccer matches are characterized by high-speed running while dribbling, passing, kicking or throwing the ball, with players required to make quick, precise movements, actions requiring multi-directional deceleration and acceleration, in addition to rapid changes of direction, all placing high demands on several physical components [11, 25]. Thus, physical stress during training and/or match play prompts morphological, metabolic, and functional adaptations that consequently enable an increase in performance [10, 36]. On the other hand, the stresses of both training and competition can cause temporary reductions in physical performance and post-match fatigue recovery, both being associated with an increased risk of musculoskeletal injury [25].

Exercise-induced muscle damage is characterized by a temporary decrease in maximal force-generating capacity, impaired physical performance, and increase in intracellular proteins in the blood, muscle soreness, and swelling in and around the involved muscle groups [18, 25, 31]. Up to 70% of soccer injuries occur in the lower limbs, typically during running, body contact, and eccentric muscle actions required when braking from sprints (deceleration) and landing from jumps, which cause a decline in physical performance lasting hours or days following a match [18, 33]. The magnitude of physical stress on the skeletal muscle system can be inferred by measuring the plasma creatine kinase (CK) and skin temperature by thermal images to determine changes in response to physiological processes or pathological reactions related to training and/or matches [18, 25]. Measuring the physical stress involving the implementation of single combined techniques is

important to develop recovery strategies that might accelerate the time to achieve full recovery as well as reduce the risk of injury [23, 26].

The development of strategies to recover from physical stress in soccer players allows a better understanding of the physiological responses and injury prevention associated with training load and match intensity. For instance, cold water immersion and contrast water therapy (88% of teams), active recovery therapy (ART) (81%), massage (78%), stretching (50%), compression garments (22%) and electrical stimulation (13%) are among the most used strategies [14, 20, 28]. In addition, nutrition and hydration were considered as very effective recovery strategies by 97% of the practitioners, while sleep was also considered as an effective recovery strategy by 95% of practitioners [27, 32]. In particular, ART consists of maintaining sub-maximal work after matches with the aim of preserving performance level between matches. There are basically 3 active recovery techniques that are applied in soccer: self-myofascial release (muscle relaxation through mobilization and loosening of the limbs in pairs); mobility and stability exercise; low-intensity aerobic activity (jogging, cycling and/or deep-water running). Thus, exercises are used as a therapeutic tool to accelerate the recovery [27, 28]. However, no study has investigated the relationship between intermittent negative pressure therapy (INPT) and post-match recovery in elite soccer players.

INPT is a technique that promotes negative pressure in the lower limbs. This device consists of a vacuum-sealing cylindrical space in which the lower body (up until the waist) of a lying subject is enclosed. In the waist region, the inner space of the device is sealed by means of a lens and within the cylindrical space, a vacuum pump generates intermittent normal and low pressure (range 28 to 52 mmHg). INPT is a therapy used in wound treatment to increase granulation tissue formation, remove edema, stimulate a cell-mediated immune response, decrease blood vessel permeability, and stimulate angiogenesis and blood flow to the wound margins [5, 8, 19, 22]. Additionally, INPT promotes an increase in foot macro- and microcirculatory flow pulsatility and mean arterial blood flow velocity in patients with peripheral arterial disease [35]. Apparently, only one study has investigated INPT after exercise-induced muscle damage therapy in elite athletes; an attenuated loss of muscle strength and power along with decreased perception of pain in the group receiving 60 minutes daily INPT therapy was reported, but no changes in biochemical markers [15]. Therefore, this study compared the effects of INPT vs. ART in the post-match physiological parameters serum CK level and skin temperature of the lower limbs in elite soccer players. Our hypothesis was that INPT would improve the recovery from physical stress (changes in biochemical markers and thermal images) because it is a method

of passive recovery, compared to the method of active recovery (ART).

Materials and methods

Participants

Participants were eligible if they were non-smokers (for the previous 3 months or more); had no cardiovascular or metabolic diseases, recent musculoskeletal injury (in the last 6 months), or pain in any region of the body; and had not used anabolic steroids, drugs or any medication with the potential to impact physical performance (self-reported).

This study included 20 healthy male elite soccer players from a club in Brazil participating in first-division national and international competitions organized by the Brazilian Soccer Confederation and South American Soccer Confederation. The participants were randomly divided into two groups to receive INPT ($n = 10$) or ART ($n = 10$). The participants' training frequency was 6.1 ± 0.5 days/week, with mean duration for each session training of 65 min, using training programs consisting of jumps, contesting possession, sprints, accelerations and decelerations. All participants performed in 2 official matches for 87.4 ± 9.3 minutes interspersed by 4 days.

This study was approved by the institutional Ethics Committee for Human Experiments (CAAE: 76189817.0.0000.5235) and was performed in accordance with national standards in sport and exercise science research. All participants signed the informed consent form.

Study design

This is a randomized, parallel arm, open label, comparative study. The sample size was determined by including all participants enrolled in the soccer matches who complied with the eligibility criteria. The intervention was conducted 24 h after a match with assessments immediately before and after the intervention and again 24 h after the intervention. Five athletes were submitted randomly to INPT 24 hours after the first match and five athletes submitted to INPT 24 hours after the second match. All measurements occurred between 8:00 and 9:00 A.M. in a quiet and temperature-controlled environment (temperature 21°C, 65% relative humidity) by a Hygro-Thermometer with Humidity Alert (Extech Instruments, Massachusetts, EUA) to allow participants to obtain their thermoneutral zone and to reduce sympathetic stress that could create artifacts [34]. The equilibration period to evaluate skin temperature was set at 15 minutes. Data collection was performed prior to scheduled training sessions by a panel of examiners blinded to each other's results. Coffee, tea and alcohol intake was prohibited for four hours before measurements. Also, the participants refrained from

physiotherapy (e.g. massage, electrotherapy, ultrasound, heat treatment, cryotherapy, hydrotherapy) before any measurements and did not apply cosmetic products to the skin to obtain meaningful readings of skin temperature by thermal images.

Anthropometric measurements

Body composition was measured following an 8-h overnight fast by bioelectrical impedance analysis using a device with built-in hand and foot electrodes (InBody 720, Biospace, Korea; inbody.com). The participants wore their normal indoor clothing and were instructed to stand bare-foot in an upright position with both feet on separate electrodes on the device's surface and with their arms abducted and both hands gripping two separate electrodes on each handle of the device.

Training load monitoring and global position system (GPS)

During two official matches, the movement of all soccer players was monitored and quantified by means of portable global position system (GPS) devices (MinimaxX, v.4.0, Catapult Innovations; Australia) operating at a sampling frequency of 10 Hz and incorporating a 100 Hz triaxial accelerometer. Each player wore a special harness enabling the device to be fitted to the upper part of his back. The GPS devices were activated 10 min prior to the start of each official match in accordance with the manufacturer's instructions. After each match, GPS files were downloaded to a computer and analyzed using the software provided by the manufacturer (Logan Plus Version 4.2.3, Melbourne, Australia). The participants' data were excluded from the analysis if they failed to complete any match due to injury and/or substitution.

The GPS devices used in the current study did not delineate forward, backward, or lateral directions, with all movement being considered universal. The indicators of the external load were as follows: 1) total distance covered; 2) frequency of efforts ≥ 18 km/h; 3) frequency of efforts ≥ 24 km/h; 4) maximal speed during matches. In addition, data obtained combining accelerations produced in three planes of body movement by means of a 100 Hz triaxial accelerometer within the GPS device were used

to classify the external training load using the player-load equation. Player-load is used as an indicator of the external load because acceleration is proportional to force and may provide a useful measure of the total load applied to a player within a match [12]. Player-load was calculated by Logan Plus software using the following equation:

$$(ay1 - ay-1)^2 + (ax1 - ax-1)^2 + (az1 - az-1)^2,$$

where ay = antero-posterior acceleration, ax = medio-lateral acceleration, and az = vertical acceleration.

Intermittent negative pressure therapy (INPT)

The INPT group was exposed to the intervention according to the manufacturer's recommendations and the protocol described in Table 1. The participants were comfortably clothed and seated for 15 minutes before the experiment started. In sequence, they were taken to the vacuum chamber with internal padding to allow insertion of both legs before sucking the air out of the bag and creating a hypobaric environment (Vacusport; Weyergans High Care, Dueren, Germany) (Fig. 1). Each therapy lasted for 30 minutes and consisted of intermittently exchanging of hypobaric pressure in the range 33 to 51 mmHg. Pressures were continuously monitored throughout the study using a calibrated pressure probe attached to the vacuum cham-

Fig. 1. Equipment utilized to intermittent negative pressure therapy (INPT)

Table 1. Description of the intermittent negative pressure therapy protocol

Phases	Time of negative pressure [s]	Intervals [s]	Exposure time of each phase [min]	Negative Pressure [mmHg]
#1	8	6	6	33
#2	6	8	6	40
#3	9	6	6	46
#4	6	9	6	51
#5	9	9	6	43

ber, which provides the values through the equipment's display.

Active recovery therapy (ART)

Participants underwent an ARC protocol for 30 minutes 24 hours after the match. ART was divided into three moments: 1) a foam roller (FR) with dimensions of 6.25" × 6.25" × 20.25" (SKLZ, CA, USA) was applied over anterior-posterior regions of the thighs and legs in both lower limbs for 4 sets of 20 s in each region of interest with a 20-s rest period between sets. The participants started at the proximal region of the thighs and legs and rolled down toward to the distal region. All participants were instructed to utilize their body mass over the thighs and legs with the help of the arms to make the movement and speed was controlled by a metronome (2 s per pass); 2) all participants conducted a 10-min mobility and stability exercise regime comprising multiple sets (i.e., 2–3 sets) from static movements to dynamic movements and finally a combination of these. The exercise program involved muscular activation of lower limb and trunk muscles using five exercises for anterior-posterior regions (i.e., prone planks; shoulder bridges; single leg deadlift with body weight; side bridges; and sidestep with elastic resistance around the thighs in a squatting position) with 10–15 s contraction time (isometric condition) and 8–10 repetitions (dynamic condition); and 3) active recovery using a cycle ergometer (60 rpm, 80 W) for 10 minutes. The ART protocol was supervised by a physical trainer with more than 10 years of experience.

Measurement of plasma CK

Plasma CK monitoring was assessed by reflectance photometry at 37°C using a previously calibrated device (Reflotron Plus; Roche, Germany) following the manufacturer's instructions. After sterilizing the finger using 70% ethyl alcohol, a disposable lancet with an automatic trigger was used to puncture the fingertip and blood was drained into heparinized capillary strips for analysis. A 32 µL sample of blood was immediately pipetted into a CK test strip and inserted into the analyzer. Absolute values of CK were used for analysis.

Measurement of skin temperature

Thermal image sequences of the lower limbs (thighs, legs, and knee) were acquired from both anterior and posterior views by a digital infrared thermo-camera (Flir Systems Inc, model T-540, USA; measurement range of 20°C to 1500°C; accuracy of ±2°C or 2%; sensitivity of ≤0.05°C), with an infrared spectral band from 7.5 to 14 microns, a refresh rate of 60 Hz and an FPA (focal plane array) of 464 × 348 resolutions for a total of 161.472 pixels. Figure 2 shows a representative thermal image from thighs and legs in both anterior and posterior views. The distance between the participants and the camera was standardized at 4 meters and the index of human skin emissivity was set to 0.98. To analyze the thermal images body regions of interest (ROI) were selected by a drawing rectangular area using the software (Smartview3.1 – Fluke, Everett, USA), which provided both the average and maximum temperatures from each ROI [25]. Selection of the thigh

Fig. 2. Thermal images of the knee, anterior (A) and posterior (B) views from thighs and legs

ROI utilized 5 cm above the upper border of the patella and groin line, whereas for the leg ROI we marked 5 cm below the lower border of the patella and 10 cm above the malleolus [25]; the knee ROI was defined around the patella.

Statistical analysis

Data were summarized as mean $\pm$ SD. Statistical analysis was initially performed using the Shapiro-Wilk normality test and the homoscedasticity test (Bartlett criterion). Student's t-test was used to assess differences in the indicators of the external load between matches (match #1 vs. match #2). Two-way analysis of variance (ANOVA) was used to test for main and interaction effects of group (levels: INPT, ART) and timing of measurement (levels: pre-intervention, post-intervention, 24 h post-intervention) for each outcome variable independently. Statistical analysis was performed with GraphPad (Prism 6.0, San Diego, CA, USA) software. The statistical significance level was set at $p < 0.05$ with Bonferroni post-hoc tests ($p < 0.003$).

Results

Table 2 shows the baseline characteristics of participants. Age, height, bodyweight and body fat were not significantly different between groups (Table 2). Student's t-test (Table 3) showed no significant between-match differences for total distance covered ($p = 0.505$),

Table 2. Baseline characteristics of the INPT and ART group

Variables	INPT	ART	P value
Age [years]	20.4 $\pm$ 3.7	21.7 $\pm$ 4.3	0.4512
Height [cm]	178.5 $\pm$ 6.2	179.5 $\pm$ 5.2	0.5285
Weight [kg]	74.3 $\pm$ 5.2	73.8 $\pm$ 6.2	0.3334
Body fat [%]	10.4 $\pm$ 4.1	10.8 $\pm$ 4.6	0.7938

Table 3. Indicators of external load during match

Variables	Match #1	Match #2	P value
Total distance covered [m]	8828 $\pm$ 1323	8419 $\pm$ 1032	0.5047
Horizontal velocity [m/min]	97.3 $\pm$ 8.4	91.4 $\pm$ 12.9	0.2775
Frequency of efforts ≥ 18 km/h	55.8 $\pm$ 19.3	42.5 $\pm$ 18.1	0.1811
Frequency of efforts ≥ 24 km/h	10.7 $\pm$ 8.5	8.2 $\pm$ 4.8	0.3222
Maximal speed [km/h]	31.6 $\pm$ 3.5	30.6 $\pm$ 2.6	0.2538
Player Load (A.U.)	840.3 $\pm$ 117.1	820.9 $\pm$ 150.2	0.3774
Player Load per minute (A.U.)	9.3 $\pm$ 1.4	9.7 $\pm$ 1.6	0.5559
Total minutes	88.6 $\pm$ 9.2	86.2 $\pm$ 10.2	0.1656

horizontal velocity ($p = 0.278$), frequency of efforts ≥ 18 km/h ($p = 0.181$), frequency of efforts ≥ 24 km/h ($p = 0.322$), maximal speed ($p = 0.254$), player load ($p = 0.377$), player load per minute ($p = 0.556$) or total minutes ($p = 0.166$).

The two-way ANOVA yielded main effects for group ($F_{1,18} = 6.969$, $p = 0.017$, $\eta^2 = 0.279$) and for timing of measurement ($F_{2,36} = 12.248$, $p < 0.001$, $\eta^2 = 0.352$), such that the CK concentration was significantly higher for ART than for INPT and decreased from pre-intervention to 24 h post-intervention. Post-hoc analysis showed that CK concentration significantly decreased from pre-intervention to 24-h post-intervention ($p < 0.001$). Most importantly, the interaction effect was significant ($F_{2,36} = 4.503$, $p = 0.018$, $\eta^2 = 0.130$), indicating that the decrease of CK from pre-intervention to 24 h post-intervention was greater in the INPT than in the ART group (Fig. 3).

Summary statistics for the effects of INPT and ART groups by timing of measurements are shown in Tables 4 and 5. The two-way ANOVA showed interaction effects group*timing for the maximum skin temperature

Fig. 3. Mean $\pm$ SD values from serum CK level pre- and post-intervention and again 24h post-intervention in elite soccer players. INPT: intermittent negative pressure therapy; ART: active recovery therapy. * $p < 0.01$ – INPT vs. ART

Table 4. Maximal values of skin temperature (°C) of thigh, leg, and knee in elite soccer players (n = 10 per group)

Body regions of interest		Pre-intervention	Post-intervention	24h Post-intervention	Main and interaction effects
Anterior thigh left					Interaction: $F_{2,36} = 4.404$, $p = 0.019$, $\eta^2 = 0.194$
	INPT	32.7 ± 0.7	32.2 ± 0.5	32.6 ± 0.7	Group: $F_{1,18} = 18.180$, $p < 0.001$, $\eta^2 = 0.503$
	ART	33.3 ± 0.8	33.7 ± 0.6	33.6 ± 0.7	Timing: $F_{2,36} = 4.404$, $p = 0.742$, $\eta^2 = 0.013$
Anterior thigh right					Interaction: $F_{2,36} = 7.542$, $p = 0.002$, $\eta^2 = 0.284$
	INPT	32.7 ± 0.8	32.1 ± 0.6	32.5 ± 0.7	Group: $F_{1,18} = 18.570$, $p < 0.001$, $\eta^2 = 0.508$
	ART	33.1 ± 0.5	33.6 ± 0.6	33.6 ± 0.7	Timing: $F_{2,36} = 1.001$, $p = 0.377$, $\eta^2 = 0.038$
Posterior thigh left					Interaction: $F_{2,36} = 1.807$, $p = 0.179$, $\eta^2 = 0.091$
	INPT	32.6 ± 0.8	32.4 ± 0.4	32.3 ± 0.5	Group: $F_{1,18} = 24.750$, $p < 0.001$, $\eta^2 = 0.579$
	ART	33.1 ± 0.7	33.3 ± 0.7	33.4 ± 0.3	Timing: $F_{2,36} = 0.051$, $p = 0.951$, $\eta^2 = 0.003$
Posterior thigh right					Interaction: $F_{2,36} = 1.749$, $p = 0.188$, $\eta^2 = 0.080$
	INPT	32.8 ± 0.6	32.3 ± 0.6	32.3 ± 0.4	Group: $F_{1,18} = 20.680$, $p < 0.001$, $\eta^2 = 0.535$
	ART	33.4 ± 0.9	33.3 ± 0.7	33.5 ± 0.4	Timing: $F_{2,36} = 1.749$, $p = 0.131$, $\eta^2 = 0.098$
Anterior leg left					Interaction: $F_{2,36} = 1.772$, $p = 0.003$, $\eta^2 = 0.249$
	INPT	32.4 ± 0.6	31.5 ± 0.5	$32.1 \pm 0.5^*$	Group: $F_{1,18} = 63.31$, $p < 0.001$, $\eta^2 = 0.779$
	ART	32.9 ± 0.6	33.1 ± 0.5	$33.2 \pm 0.3^*$	Timing: $F_{2,36} = 3.004$, $p = 0.062$, $\eta^2 = 0.107$
Anterior leg right					Interaction: $F_{2,36} = 7.697$, $p = 0.002$, $\eta^2 = 0.258$
	INPT	32.6 ± 0.7	31.5 ± 0.5	$32.2 \pm 0.4^*$	Group: $F_{1,18} = 79.750$, $p < 0.001$, $\eta^2 = 0.816$
	ART	33.0 ± 0.7	33.3 ± 0.5	$33.4 \pm 0.3^*$	Timing: $F_{2,36} = 4.143$, $p = 0.024$, $\eta^2 = 0.139$
Posterior leg left					Interaction: $F_{2,36} = 2.143$, $p = 0.132$, $\eta^2 = 0.086$
	INPT	32.3 ± 0.7	31.4 ± 0.7	$32.2 \pm 0.5^*$	Group: $F_{1,18} = 13.930$, $p = 0.002$, $\eta^2 = 0.436$
	ART	32.5 ± 0.7	32.5 ± 0.8	$32.9 \pm 0.4^*$	Timing: $F_{2,36} = 4.876$, $p = 0.013$, $\eta^2 = 0.195$
Posterior leg right					Interaction: $F_{2,36} = 4.200$, $p = 0.023$, $\eta^2 = 0.153$
	INPT	32.4 ± 0.6	31.5 ± 0.6	$32.2 \pm 0.4^*$	Group: $F_{1,18} = 18.660$, $p < 0.001$, $\eta^2 = 0.436$
	ART	32.6 ± 0.4	32.6 ± 0.8	$32.8 \pm 0.4^*$	Timing: $F_{2,36} = 5.243$, $p = 0.010$, $\eta^2 = 0.191$
Anterior knee left					Interaction: $F_{2,36} = 1.000$, $p = 0.378$, $\eta^2 = 0.049$
	INPT	31.8 ± 0.9	31.5 ± 0.6	31.7 ± 0.5	Group: $F_{1,18} = 20.070$, $p < 0.001$, $\eta^2 = 0.527$
	ART	32.4 ± 0.8	32.4 ± 0.5	32.8 ± 0.5	Timing: $F_{2,36} = 1.514$, $p = 0.234$, $\eta^2 = 0.074$
Anterior knee right					Interaction: $F_{2,36} = 0.733$, $p = 0.487$, $\eta^2 = 0.037$
	INPT	31.6 ± 1.0	31.4 ± 0.8	31.6 ± 0.9	Group: $F_{1,18} = 15.000$, $p < 0.001$, $\eta^2 = 0.455$
	ART	32.3 ± 0.9	32.4 ± 0.5	32.8 ± 0.4	Timing: $F_{2,36} = 0.902$, $p = 0.415$, $\eta^2 = 0.046$

INPT: intermittent negative pressure therapy; ART: active recovery therapy. Post-hoc analysis: * $p < 0.003$ compared to pre-intervention.

measured at the anterior thigh (left: $F_{2,36} = 4.404$, $p = 0.019$, $\eta^2 = 0.194$; right: $F_{2,36} = 7.542$, $p = 0.002$, $\eta^2 = 0.284$), anterior leg (left: $F_{2,36} = 1.772$, $p = 0.003$, $\eta^2 = 0.249$; right: $F_{2,36} = 7.697$, $p = 0.002$, $\eta^2 = 0.258$), as well as posterior right leg ($F_{2,36} = 4.200$, $p = 0.023$, $\eta^2 = 0.153$). In general, the maximal skin temperature for those regions were larger for the ART than the INPT group but also it tended to increase in the ART but to decrease in the INPT group throughout the timing of measurements. In the absence of statistical evidence for interaction effects, significant

main effects for group were observed for the maximum skin temperature measured at the posterior thigh (left: $F_{1,18} = 24.750$, $p < 0.001$, $\eta^2 = 0.579$; right: $F_{1,18} = 20.680$, $p < 0.001$, $\eta^2 = 0.535$), posterior left leg ($F_{1,18} = 13.930$, $p = 0.002$, $\eta^2 = 0.436$), and knee (left: $F_{1,18} = 20.070$, $p < 0.001$, $\eta^2 = 0.527$; right: $F_{1,18} = 15.000$, $p < 0.001$, $\eta^2 = 0.455$). Conversely, significant main effects for timing of measurement were observed only for the maximum skin temperature at the posterior left leg ($F_{2,36} = 4.876$, $p = 0.013$, $\eta^2 = 0.195$).

Table 5. Mean values of skin temperature (°C) of thigh, leg, and knee in elite soccer players (n = 10 per group)

Body regions of interest		Pre-intervention	Post-intervention	24h Post-intervention	Main and interaction effects
Anterior thigh left					Interaction: $F_{2,36} = .900, p = .415, \eta^2 = .045$
	INPT	31.6 ± 0.7	31.4 ± 0.8	31.8 ± 0.7	Group: $F_{1,18} = 18.250, p < .001, \eta^2 = .503$
	ART	32.3 ± 0.9	32.6 ± 0.3	32.7 ± 0.4	Timing: $F_{2,36} = 1.265, p = .294, \eta^2 = .063$
Anterior thigh right					Interaction: $F_{2,36} = 1.249, p = .299, \eta^2 = .061$
	INPT	31.6 ± 0.7	31.4 ± 0.8	31.7 ± 0.8	Group: $F_{1,18} = 15.220, p < .001, \eta^2 = .458$
	ART	32.3 ± 1.1	32.6 ± 0.5	32.7 ± 0.4	Timing: $F_{2,36} = 1.211, p = .310, \eta^2 = .059$
Posterior thigh left					Interaction: $F_{2,36} = 1.298, p = .285, \eta^2 = .066$
	INPT	31.8 ± 0.9	31.6 ± 0.8	31.8 ± 0.7	Group: $F_{1,18} = 11.800, p = .003, \eta^2 = .396$
	ART	32.2 ± 0.7	32.5 ± 0.4	32.6 ± 0.3	Timing: $F_{2,36} = .494, p = .614, \eta^2 = .025$
Posterior thigh right					Interaction: $F_{2,36} = 6.725, p = .003, \eta^2 = .199$
	INPT	32.4 ± 0.6	31.4 ± 0.8	31.7 ± 0.8	Group: $F_{1,18} = 4.889, p = .040, \eta^2 = .214$
	ART	32.4 ± 0.8	32.3 ± 0.7	32.6 ± 0.4	Timing: $F_{2,36} = 9.045, p < .001, \eta^2 = .268$
Anterior leg left					Interaction: $F_{2,36} = 3.178, p = .034, \eta^2 = .131$
	INPT	31.7 ± 0.8	30.7 ± 0.7	$31.5 \pm 0.5^*$	Group: $F_{1,18} = 28.550, p < .001, \eta^2 = .613$
	ART	32.1 ± 0.9	32.1 ± 0.4	$32.5 \pm 0.3^*$	Timing: $F_{2,36} = 6.740, p = .003, \eta^2 = .237$
Anterior leg right					Interaction: $F_{2,36} = 1.933, p = .159, \eta^2 = .070$
	INPT	31.6 ± 0.9	30.8 ± 0.8	$31.6 \pm 0.6^*$	Group: $F_{1,18} = 28.680, p < .001, \eta^2 = .614$
	ART	32.3 ± 0.7	32.1 ± 0.5	$32.6 \pm 0.3^*$	Timing: $F_{2,36} = 7.834, p < .001, \eta^2 = .282$
Posterior leg left					Interaction: $F_{2,36} = 1.140, p = .331, \eta^2 = .047$
	INPT	31.5 ± 0.7	30.9 ± 0.7	31.4 ± 0.6	Group: $F_{1,18} = 15.800, p < .001, \eta^2 = .467$
	ART	31.9 ± 0.6	31.7 ± 0.7	32.3 ± 0.3	Timing: $F_{2,36} = 4.883, p = .013, \eta^2 = .203$
Posterior leg right					Interaction: $F_{2,36} = 1.733, p = .191, \eta^2 = .066$
	INPT	31.6 ± 0.8	30.7 ± 0.6	$31.4 \pm 0.7^*$	Group: $F_{1,18} = 15.670, p < .001, \eta^2 = .465$
	ART	32.0 ± 0.6	31.7 ± 0.7	$32.2 \pm 0.4^*$	Timing: $F_{2,36} = 6.602, p = .004, \eta^2 = .251$
Anterior knee left					Interaction: $F_{2,36} = .396, p = .676, \eta^2 = .017$
	INPT	30.4 ± 1.0	29.9 ± 0.8	30.6 ± 1.0	Group: $F_{1,18} = 10.750, p = .004, \eta^2 = .374$
	ART	31.1 ± 1.1	31.0 ± 0.7	31.7 ± 0.5	Timing: $F_{2,36} = 5.409, p = .009, \eta^2 = .227$
Anterior knee right					Interaction: $F_{2,36} = .244, p = .785, \eta^2 = .012$
	INPT	30.4 ± 1.2	30.0 ± 0.7	30.5 ± 1.1	Group: $F_{1,18} = 11.610, p = .003, \eta^2 = .392$
	ART	31.3 ± 1.2	31.2 ± 0.8	31.7 ± 0.5	Timing: $F_{2,36} = 2.420, p = .103, \eta^2 = .117$

INPT: intermittent negative pressure therapy; ART: active recovery therapy. Post-hoc analysis: * $p < 0.003$ compared to pre-intervention.

The two-way ANOVA showed interaction effects group by timing for the mean skin temperature measured at the posterior right thigh ($F_{2,36} = 6.725, p = 0.003, \eta^2 = 0.199$) and anterior left leg ($F_{2,36} = 3.178, p = 0.034, \eta^2 = 0.131$). In general, the mean skin temperature for those regions were larger for the ART than the INPT group and tended to decrease from pre- to post-intervention and to increase again from post- to 24 h post-intervention. In the absence of statistical evidence for interaction effects, significant main effects for group were observed for the

mean skin temperature measured at the anterior thigh (left: $F_{1,18} = 18.250, p < 0.001, \eta^2 = 0.503$; right: $F_{1,18} = 15.220, p < 0.001, \eta^2 = 0.458$), posterior left thigh ($F_{1,18} = 11.800, p = 0.003, \eta^2 = 0.396$), anterior right leg ($F_{1,18} = 28.680, p < 0.001, \eta^2 = 0.614$), posterior leg (right: $F_{1,18} = 15.800, p < 0.001, \eta^2 = 0.467$; left: $F_{1,18} = 15.670, p < 0.001, \eta^2 = 0.465$), and knee (right: $F_{1,18} = 10.750, p = 0.004, \eta^2 = .374$; left: $F_{1,18} = 11.610, p = 0.003, \eta^2 = 0.392$). Conversely, significant main effects for timing of measurement were observed only for the mean skin temperature at the

posterior left leg ($F_{2,36} = 4.883$, $p = 0.013$, $\eta^2 = 0.203$) and anterior left knee ($F_{2,36} = 5.409$, $p = 0.009$, $\eta^2 = 0.227$).

Discussion

This study compared the effects of INPT vs. ART in post-match physiological parameters serum CK level and skin temperature of the lower limbs in elite soccer players. Our findings support the original hypothesis that INPT would improve the recovery from physical stress (physiological changes in biochemical markers and thermal images). No studies were found that evaluated the impact of INPT on serum CK level and profile of thermal images in elite soccer players 24 h and 48 h after a match. Notable results from the present study showed that INPT contributed a decrease in serum CK level of 62.4% immediately after the intervention and 61% at 24 h after the intervention compared to ART. On the other hand, the major finding herein was that all ROI investigated in this study showed a significant decrease of the thermal images immediately after the intervention and 24 h after the intervention using INPT compared to ART (Tables 4 and 5). The present study also revealed no significant difference between sides in lower limbs (left vs. right), which suggests that volunteers had the same homogeneity status. Different recovery strategies such as hydrotherapy, compression garments, and active recovery are now routinely implemented by team sports athletes after a competition [14]. However, considering that CK levels and skin temperature are increased after soccer matches by climate change, grass height, stress, and high muscle fiber recruitment during matches, we can accept our hypothesis that INPT improves neuromuscular recovery after a match.

Increased intensity of efforts leads to muscle cell disturbance and delays the appearance of a CK serum peak compared to less disruption and may be linked to the time course of inflammation [7]. Hence, intense exercise contributes to damage within the structure of skeletal muscle cells at the level of the sarcolemma, alters the muscle cell permeability, causes myofibrillar disorganization and loss of Z-disc integrity and leads to cellular protein leakage, ultimately increasing the serum activity of many enzymes such as an increase in total CK [9, 24]. Thus, greater recruitment of fast-twitch fibers during high-intensity exercise is reported to contribute to an increase in serum CK [24] and rating of perceived exertion (RPE) [13]. On the other hand, some studies demonstrate that an increased lower limb force is related to reduced levels of CK >48 h post-match and full recovery of serum CK levels at 72 h post-match [13, 29]. Our results verified that serum CK levels in INPT showed a reduction of 12.9% and 49.5% immediately post-intervention and 24 h post-intervention, respectively, compared to 24 h post-match. However, ART showed an increase of 21% in the serum CK levels

immediately post-intervention and a decrease of 10% 24 h post-intervention in relation to values of 24 h post-match.

Contrary to our results, another study induced muscle damage using 50 drop jumps and 50 legs curls which observed a peak increase in CK at 96 hours compared to the peak 24 hours after the exercises in control and INPT groups [15]. This behavior of serum CK level may be related to the population of the study as they investigated healthy young female adults involved in moderate physical activity (not athletes) and therapy lasted for 60 minutes with hypobaric pressure variation of 4.5 mmHg at 45 mmHg every 20 seconds [15]. In addition, a strong relationship has been observed whereby estrogen could affect muscle recovery [37]. However, INPT could improve recovery by limiting the loss in muscle strength and power and limiting the presence of pain [15]. Furthermore, some studies showed that ART had no effects on the recovery pattern of physical performance markers [3, 4]. A mechanism proposed for improvement of the recovery process using INPT involves reduction in the structural damage associated with leukocyte infiltration, consequently stabilizing the cell membrane during the process of repair, which explains the lower response of CK (the release of CK is attenuated as a result of negative pressure), and the greater venous return could improve the removal of myofibrillar proteins and other products due to the greater venous return [7, 21, 24, 30].

Considering that CK levels increase after soccer matches, applying two methods for muscle activity assessment are more accurate [17]. Thus, the use of thermal images combined with serum CK level can together determine muscle damage, because only biochemical markers do not show the anatomical location of the muscle injury and both methods can provide rapid feedback for coaches during training phases. However, the elevated skin temperatures found 24 to 72 hours after exercise in all participants is possibly the result of higher blood flows in muscle due to inflammation, and tissue damage repair [2]. Indeed, thermal image changes can be related to higher workout intensity and morphological changes in muscle tissue by modifying the cross-sectional area of the muscle [16, 38]. This response reveals that trained participants have a higher cutaneous blood flow than others by several physiological changes (e.g., increased muscle metabolism, anaerobic energy reserve, density of capillaries, nerve conduction rate and morphological changes in muscle tissue by modifying the cross-sectional area of the muscle) [1, 16, 25, 38]. A recent study published by our research group showed that thermal symmetry of the lower limbs was similar between the sides of the body at rest in elite soccer players, being identical in shape and size [25]. In the present study, evaluated elite soccer players showed contralateral thermal symmetry in both groups (INPT and ART) and all the ROI were not greater than 0.2°C. On the other hand, our results showed differences between groups in maximal and mean

values $> 0.7^{\circ}\text{C}$ immediately post-intervention and 24 h post-intervention in front and back lower limbs in which a differences in thermal image ($>0.7^{\circ}\text{C}$) can be associated with structural or physiological abnormalities that contribute to a decrease in the quadriceps power [25, 39]. These differences $>0.7^{\circ}\text{C}$ in the skin temperature between elite soccer players (INPT vs. ART) possibly by INPT contribute to the process of recovery from the increased blood flow in the microcirculation, reduction of edema, removal of exudate, cell proliferation and angiogenesis, which can lead to faster neuromuscular recovery, reduced muscle tension, and reduced pain sensation [6, 15].

A limitation of this type of investigation could be the specific position of the soccer players, climatic conditions and condition of the grass during the soccer match since these parameters could influence serum CK level, skin temperature measurements, and autonomic response. While our sample was homogeneous, the sample size was not large enough to provide variations in physiological condition to specific positions. Future research should elucidate the influence of the external training load in the biochemical markers during training and/or matches in elite soccer players.

Conclusions

Serum CK level and skin temperature of lower limbs showed better recovery up to 24 h post-intervention using INPT in elite soccer players. It is recommended to use such a passive recovery strategy over ART the day after a game as the latter might be counterproductive to the recovery process.

Practical applications

- INPT is a new approach to design and optimize the time of recovery in elite soccer players. Thus, coaches should consider these data when planning workouts and training after matches and monitor recovery to ensure optimal performance during competitions.
- INPT may have beneficial effects for post-matches recovery because of the decreasing serum CK level and skin temperature of lower limbs.
- We recommend that future studies should be performed to clarify the mechanisms associated with the recovery process in elite soccer players.

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