Effect of alterations in whole-body cryotherapy (WBC) exposure on post-match recovery markers in elite Premier League soccer players

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ABSTRACT: The use of whole-body cryotherapy (WBC) as a recovery intervention is prevalent amongst elite soccer players. However, there is a distinct lack of data available around chronic WBC use and post-match recovery markers in elite soccer. The aim of this study was to investigate the impact of different levels of WBC exposure on subjective and objective measures of post-match recovery in elite soccer players during a chronic exposure period. Sixteen male senior professional outfield soccer players participated in this study over two seasons. K means cluster analysis was used to classify low (-114 ± 2°C for 133 ± 2 s), medium (-121 ± 1°C for 173 ± 2 s) and high (-133 ± 1°C for 181 ± 2 s) cryotherapy exposure indexes (CEI). Salivary markers (immunoglobulin A (IgA) and alpha amylase (AA)) and subjective wellness scores (perceived fatigue, sleep quality, general muscle soreness and stress) were collected post-match across both seasons. Training load (session-RPE) was collected and used as a covariate to control for the load amongst groups. No differences were seen in perceived measures of wellness and salivary AA. Significantly lower IgA concentrations were observed in the medium CEI group (255 ± 32 $\mu g \cdot ml^{-1}$) compared to the low (328 ± 38 $\mu g \cdot ml^{-1}$) and high (306 ± 32 $\mu g \cdot ml^{-1}$) groups. Therefore, increasing the level of chronic WBC exposure appears to have no additional benefit on subjective recovery and alpha amylase response post-match. However, there appears to be an optimal chronic WBC dose with regards to IgA response.

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INTRODUCTION

Soccer is a highly demanding intermittent sport that involves fluctuations between low and high intensity activities [1]. At the elite level, soccer players typically cover around 11–13 km per match dependent on position, with central midfielders covering the highest and central defenders the lowest distance, respectively [2]. Of this overall distance covered, around ~1150 m is run at speeds above 20 $km.h^{-1}$, with around ~60 sprints performed again dependent on the positional role within the team [3]. Overall players perform more than ~1200 unpredictable changes in activity, which also comprises of ~700 turns and 30–40 tackles and jumps [4]. Such physical demands can result in significant fatigue, with reductions in muscle glycogen content, neuromuscular function and muscle damage markers up to 72 hours post-match [5–8]. For elite teams, this timeline of recovery can become a coaching planning issue when multiple matches are played in a short duration (e.g. 2–3 matches in a 1-week period). Therefore, sport science practitioners look to utilise interventions to augment the recovery process, enabling players to undertake increased training loads and improve match readiness [9].

Whole-body cryotherapy (WBC) is a recovery method that involves exposure to extreme cold temperatures (-110 to -195°C) over a short period of time (1–3 minutes) in a temperature-controlled chamber. Such temperatures induce vasoconstriction, leading to an increase in central blood pressure and subsequent reduction in sympathetic nerve activity [10]. WBC has been suggested to improve muscular enzyme recovery responses [11, 12], reduce haemolysis [11] and improve subjective perceptions of recovery [13]. It has been previously suggested that several variables may influence the physiological response to WBC, such as exposure duration, temperature and frequency of sessions [14]. Previous research has suggested that at least two minutes of exposure is required to significantly reduce skin temperature [15, 16]. There is currently a lack of research investigating the impact of varying levels of chronic WBC exposure. Westerlund et al. [17] found similar changes in blood pressure following exposure

at -110°C three times per week during a three month period. Louis et al. [14] recently reported a temperature of at least -110°C is required to stimulate the autonomic nervous system through parasympathetic drive when undertaking WBC across five consecutive days. There is currently limited research into the influence of chronic WBC exposure in sporting populations [12, 18]. In addition, elite soccer players typically expose themselves to WBC during a full competitive season (i.e. 9–10 months), thus the effect of such chronic exposure on post-match recovery has yet to be investigated.

The aim of this study was to investigate the impact of different levels of WBC exposure based on cryotherapy exposure index (CEI: cryotherapy temperature and duration) on subjective and objective measures of post-match recovery in elite soccer players during a chronic exposure period.

MATERIALS AND METHODS

Participants

Sixteen male senior professional outfield soccer players (age: 27 ± 4 years; height: 179 ± 5 cm; weight: 76 ± 6 kg) from an English Premier League team took part in this study. Data collection arose as a condition of the player's usual daily duties as a professional player which were routinely measured over the course of the competitive season. The study conformed to the recommendations of the Declaration of Helsinki and was approved by the local university ethics committee. All player's data was anonymised prior to analysis to ensure confidentiality and informed consent was provided.

Experimental Design

In an observational study design, WBC exposure and post-match recovery data were collected across both the 2016–17 (38 matches) and 2017–18 (31 matches) English Premier League seasons. Data was only included during the in-season period across the macrocycle (i.e. exclusion of pre-season data). All players provided data across both seasons and were considered regular starters by the club (i.e. starting 11 players and/or regular rotation from the substitutes bench). In addition, the same head coach and technical staff were employed across both seasons which provided consistency within the coaching philosophy adopted during training practices. WBC exposure was provided to players following match play across the weekly microcycle. This differed dependent on whether the team were playing a home or away fixture. Following home games players were encouraged to use WBC just before they were leaving the stadium, which varied from 45–90 mins following the cessation of the match. Exposure following away games was largely dependent on the location, and method of team travel. Players were encouraged to use WBC as soon as they returned to the training ground following an away match, which meant a less standardised window of exposure. Using k means cluster analysis, three clusters (k = 3) were determined for analysis and classified into low (-114 ± 2°C for 133 ± 2 s), medium (-121 ± 1°C for 173 ± 2 s) and high (-133 ± 1°C for 181 ± 2 s) CEI groups to offer more practically intuitive and functionally relevant findings.

Data Collection and Interventions

Whole-Body Cryotherapy

Players were exposed to WBC using a whole-body cryotherapy chamber (CryoAction, London, UK). Recommended club protocol suggested players spent 30 seconds in a pre-cooling chamber set to -30°C, followed by 150 seconds of WBC exposure at -130°C. However, temperature and duration were often adjusted by the players and/or medical team. The CEI was calculated as the cryotherapy temperature multiplied by the duration of exposure in seconds and used to split the players into exposure groups as described previously. All exposure data was collected by a member of the sports science or medical staff using an electronic device and logged on a secure athlete database. Before entering the chamber, players were asked to remove glasses, contact lenses and any jewellery or piercings. During exposure, players were encouraged to wear a pair of shorts and nothing above the waist, although some players kept a t-shirt/long sleeve shirt on. Players also wore gloves, dry socks and shoes, a hat covering the ears and a mask to protect the nose and mouth. The WBC was also available to players for 45 minutes following the cessation of training. The protocol was dependent on the training and match scheduling of that week, with players deciding the frequency of their exposure.

Post-Match Recovery Measures

Wellness Questionnaire

A questionnaire [19] was adopted to assess indicators of self-reported player wellness using a 7- point scale (with 1 representing "very, very good" and 7 representing "very, very poor"). The questionnaire was composed of 4 questions related to perceived fatigue, sleep quality, general muscle soreness and external stress levels. The data was collected using an iPad application to ensure no influence of scores amongst the other players. All wellness scores were collected in the morning on arrival prior to training and monitored on a daily basis.

Salivary Markers

Passive saliva samples were collected from players approximately 40 hours after a competitive match, as part of a whole squad screening protocol prior to the first training session following a match. Sampling was kept to a consistent 10-minute time frame, as players were assigned specific group start times which were adhered to throughout the duration of the study. The players were instructed not to eat or drink any substance other than water prior to giving the sample, and passive samples were collected using an IPRO (Soma Bioscience, Wallingford, UK) oral fluid collector (OFC) kit. Each player was instructed to place the OFC in their mouth which collected 0.5ml of saliva, notification of sufficient saliva volume was provided by the handle of the swab changing colour. The swab was

then placed in an IPRO buffer solution, and the sample was shaken for 60 seconds to mix the swab and buffer solution. Two drops of the buffer mix were then applied to a sample window on a lateral flow device (LFD) cartridge, which was left to stand for 10 minutes before being placed in an LFD reader for analysis. The LFD has previously been validated against ELISA analysis ($r = 0.89$, $p < 0.01$ and CV = 9.4%) (unpublished observations). After approximately 20 seconds of analysis the LFD gave a quantitative value depicting the intensity of both immunoglobulin A (IgA) and alpha amylase (AA).

Training Load Quantification

Training load was quantified across the study using both total session minutes and the session rating of perceived exertion (session-RPE) determined using the CR10 scale of Foster et al. [20]. Session-RPE scores were collected approximately 30-minutes post-session and multiplied by the session duration to calculate the magnitude (termed "load"). The duration included the warm-up and main training session periods within each training session. Both training and match days were included in the load calculations.

Statistical Analysis

K means Cluster analysis

K means cluster analysis was used to determine different exposure groups based on CEI data. The k means algorithm is an unsupervised machine learning clustering technique which aims to divide the entire data set into groups based on patterns in the data. K-means clustering is an iterative process whereby the distances between input data and a number of randomly allocated seeds (k) in the data space are determined. This results in k temporary clusters of data each allocated to each seed. For the newly formed temporary clusters the mean of the data contained within the cluster is calculated, thus forming a new centroid; the distance of the data points in the data space to the new temporary centroids is then re-calculated. This process of re-defining the cluster centroids is repeated until the change in positions of the cluster centroids converges on a constant location [21]. Three clusters ($k = 3$) were determined for analysis to align with the traditional classification of low, medium, and high cryotherapy exposure groups to offer more clinically intuitive and functionally relevant findings. To assess the validity of this decision, internal cluster validity for $k = 1–8$ solutions were tested.

Variance components and linear mixed models

Exploratory data analysis was initially carried out to assess the assumptions of the linear mixed model (LMM), with none of the current variables violating these assumptions. A LMM was utilised to overcome the assumption of independence, and also because of the flexibility that this method has in accounting for the altering sample sizes between groups with repeated measures [22]. All models began as a null and were progressed to more complex parsimonious hierarchical models. A basic variance components model was initially executed to calculate the intraclass correlation (ICC) of the random factors of individual player to determine if it contributed significant variance to the dependant variable. Given the large sample sizes, Wald Z statistics were utilised to test the null hypothesis that the population variance is zero, if rejected the proposed random factors were included in subsequent larger models. The covariance structure of the random factors was set to variance components in all models. Model fit was assessed using Akaike's information criterion (AIC). For each dependant variable, AIC revealed the model that best fit the data utilised the first order auto-regressive (AR-1) repeated covariance structure for the repeated measures of exposure group. Due to the current dependent variables comprising wellness and salivary stress measures, RPE-based load was used as a covariate to control for the potentially confounding issue of different session loads on assessing the intervention response. All models estimated parameters using the maximum likelihood method. Where appropriate, Bonferroni adjusted post hoc analyses and the inclusion of 95 % confidence intervals (C.I.) of the differences were reported. All statistical procedures were carried out using IBM SPSS Statistics (Version 25, Chicago, IL, USA), with two-tailed significance being accepted at $p < 0.05$. All data is presented as mean ± SD unless otherwise stated.

RESULTS

K means cluster analysis

Utilising $k = 3$ and the exposure index data, the k-means model solution converged was achieved after eight iterations, with cluster centres calculated after all data points had been assigned to a given cluster (Table 1). For other K solutions, convergence was either not achieved, or the number of clusters were deemed not optimal based on visual inspection of an elbow plot of the Akaikes information criterion (AIC) of the auto clustering.

Variance Components model

Table 2 depicts the ICC's (%) of the random factors accounted for in the LMM. Except for both the AA and perceived sleep quality data, the individual player contributed significant variance to all dependant variables and was subsequently included in the larger hierarchical models.

TABLE 1. The number of observations and cluster centres observed from the K means cluster analysis for each exposure group.

	High	Medium	Low
No. of observations in each cluster	266	284	136
K means Cluster centres (au)	23798.1	20522.3	14476.8

TABLE 2. The ICC's (%) of the random factor of individual player for each of the dependant variables.

Dependent Variable	*Player (%)*
Sleep (au)	7.1*
Fatigue (au)	17.4*
Stress (au)	36.4*
MS (au)	9.6*
IgA ($\mu g \cdot ml^{-1}$)	27.6*
AA ($\mu g \cdot ml^{-1}$)	8.6*

*Represents significant determinant of variance within the LMM ($p < 0.05$).

The LMM identified a significant main effect for the different CEI groups for the RPE load ($P < 0.001$), with significantly higher RPE based load for the high exposure group (521 ± 16 au) when compared to both the medium (481 ± 15 au; 95 % CI = 8 to 71 au) and low groups (451 ± 19 au; 95 % CI: 25 to 114 au). Based on these data, RPE-based load was used as a covariate to control for the potentially confounding issue of different session loads on assessing the intervention response.

LMM outputs

The LMM identified a significant main effect for the different CEI groups for the Cryotherapy time ($P < 0.001$) and temperatures ($P < 0.001$), with significantly lower temperature and higher time values recorded for the high exposure group when compared to the medium and low exposure groups (Table 3). Significantly higher time ($P < 0.001$) and lower temperature ($P < 0.001$) values were also observed for the medium exposure group when compared to the low exposure group.

There were no main effects for the different CEI groups for measures of perceived sleep quality ($P = 0.554$), perceived fatigue ($P = 0.459$), external stress ($P = 0.669$), muscle soreness ($P = 0.248$) and AA concentration ($P = 0.348$). There was however a main effect for CEI observed for measures of IgA concentrations ($P = 0.009$), with post hoc pairwise comparisons identifying significantly lower values recorded in the medium exposure group when compared to both the low ($P = 0.037$) and high ($P = 0.037$) exposure group. No differences were observed between the other groups (Table 4).

DISCUSSION

The present study revealed that increasing the amount of WBC exposure had no additional benefit on subjective recovery and alpha amylase response post-match. However, there appears to be an optimal dose with regards to IgA response, with the medium exposure

TABLE 3. The Cryotherapy temperature and exposure time is also presented for each exposure group.

	High	Medium	Low
Cryotherapy temperature (°c)	-133 ± 1 95%CI = -15 to -11[a] 95% CI = -23 to -16[b]	-121 ± 1 95%CI = -10 to -4[b]	-114 ± 1
Cryotherapy time (s)	181 ± 2 95% CI = 5 to 11[a] 95% CI = 43 to 52[b]	173 ± 2 95% CI = 36 to 44[b]	133 ± 2

[a] and [b] denote significant differences with the medium and low exposure groups respectively. 95% confidence intervals presented for significant main effects.

TABLE 4. The influence of CEI group on the subjective wellness and salivary measures.

	High	Medium	Low
Sleep (au)	1.9 ± 0.4	1.9 ± 0.4	1.9 ± 0.5
Fatigue (au)	2.0 ± 0.7	2.0 ± 0.7	2.0 ± 0.8
Stress (au)	1.5 ± 1.0	1.5 ± 0.9	1.5 ± 1.0
Muscle Soreness (au)	2.1 ± 0.6	2.1 ± 0.6	2.2 ± 0.8
Immunoglobulin A ($\mu g \cdot ml^{-1}$)	306 ± 32 95% CI = -99 to -2	255 ± 32*	328 ± 38 95% CI = -144 to 3
Alpha Amylase ($\mu g \cdot ml^{-1}$)	494.5 ± 39.8	527.7 ± 39.6	434.4 ± 62.2

* denotes significant differences with the high and low exposure groups. 95% confidence intervals presented for significant main effects.

group observing a reduction post-match compared to high and low exposure groups.

The use of WBC is now commonplace within elite soccer, but the efficacy of long-term chronic use for post-match recovery enhancement is unknown to practitioners. We present novel data on an elite group of English Premier League soccer players over a chronic two-year period of monitoring with consistency in playing and coaching staff. Our findings revealed no significant difference in the majority of recovery measures post-match across the three exposure groups. There is currently a lack of published data available on longitudinal WBC use in sporting populations for direct comparison. The mean temperature of the three exposure groups were -133°C (high), -121°C (medium) and -114°C (low) in the present study. Louis et al. [14] recently investigated the effects of varying WBC exposure temperatures on parasympathetic activity, including -10°C, -60°C and -110°C, across five consecutive days. The authors revealed that only the lowest temperature (-110°C) induced a significant increase in parasympathetic activity. Therefore, as all of the groups in the present study had a mean exposure temperature below -110°C, this suggests an optimal temperature dose when exposing over a chronic time period. However, there needs to be significantly more research investigating the effects of longitudinal WBC use in sporting populations before any definitive conclusions can be made. Indeed, Louis et al. [14] also found lower parasympathetic responses to WBC over consecutive days when compared to day one, suggesting a possible habituation of WBC amongst participants. Westerlund et al. [17] found similar changes in blood pressure following exposure at -110°C three times per week during a three month period which also supports this notion. Therefore, our present data suggests that the adage that 'more is better' may not be the case for WBC use within elite soccer over a chronic period for post-match recovery.

An interesting finding from the present study was the significant reduction in salivary IgA concentrations post-match in the medium CEI group compared to both the high and low groups. This data would suggest an optimal level exists for chronic WBC exposure in elite soccer players with regards to their post-exercise immune response, with either higher or lower doses more appropriate over time. A reduction in salivary IgA is seen as potentially detrimental to performance due to suppression on the immune system [23]. This may lead to potential time-loss illnesses which can have a significant impact on squad availability, particularly during congested fixture periods such as the winter period in the English Premier League [23]. Salivary IgA has regularly been seen to be reduced following exercise in elite athletes, but levels return to baseline with adequate rest and recovery [24]. The saliva samples in the present study were taken at approximately 40 hours post-match, prior to the first training session for the next weekly training microcycle. Practically our data suggests that players with chronic medium CEI exposure would have potentially commenced the week with reduced IgA concentrations. Therefore, players would be encouraged to alter their WBC exposure levels to either increase or decrease in line with our CEI groups, or potentially face the consequence of reducing their training load to compensate for reduced IgA levels [25].

Due to the applied nature of this observational study, there are several limitations that must be noted. The lack of control group available within this elite sporting population means that the data cannot distinguish whether WBC as a recovery method itself works in elite soccer players. However, we are able to identify different exposure levels which is useful for soccer practitioners who seek advice around what level of exposure would be best suited within this population. Secondly, as recovery measures were only collected approximately 40 hours post-match, this limits our understanding of the overall recovery kinetics during this period. Future work should look to investigate the time course response (i.e. immediately post, 1h, 12h, 24, 48h and 72h) post-match with players who are habitually exposed to WBC. Despite these limitations, the present study provides rare novel recovery data in elite level soccer players across a significant longitudinal period.

CONCLUSIONS

The aim of the present study was to investigate the impact of different levels of WBC exposure on subjective and objective measures of post-match recovery in elite soccer players during a chronic exposure period. The findings revealed that increasing the amount of WBC exposure had no additional benefit on subjective recovery and alpha amylase response post-match. However, there appears to be an optimal chronic WBC dose with regards to IgA response, with the medium exposure group observing a reduction post-match compared to high and low exposure groups. Based on our findings, we would recommended practitioners employ the low or high WBC exposure levels highlighted in our study for use with elite soccer players. Further research into the efficacy of WBC as a recovery intervention over a chronic period is warranted, particularly investigating the time-course response post-match and the impact on subsequent training practices.

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Conflict of Interest

The authors declare no conflict of interest.

REFERENCES

1. Bangsbo J. The physiology of soccer--with special reference to intense intermittent exercise. Acta Physiol Scand Suppl. 1994;619:1–155.
2. Bradley PS, Sheldon W, Wooster B, Olsen P, Boanas P, Krustrup P. High-intensity running in English FA Premier League soccer matches. J Sports Sci. 2009;27(2):159–68.
3. Barnes C, Archer D, Hogg B, Bush M, Bradley P. The Evolution of Physical and Technical Performance Parameters in the English Premier League. Int J Sports Med. 2014;35(13):1095–100.
4. Bloomfield J, Polman R, O'Donoghue P. Physical demands of different positions in FA Premier League soccer. J Sport Sci Med. 2007;6(1):63–70.
5. Krustrup P, Ørtenblad N, Nielsen J, Nybo L, Gunnarsson TP, Iaia FM, et al. Maximal voluntary contraction force, SR function and glycogen resynthesis during the first 72 h after a high-level competitive soccer game. Eur J Appl Physiol. 2011;111:2987–95.
6. Nedelec M, McCall A, Carling C, Legall F, Berthoin S, Dupont G. The influence of soccer playing actions on the recovery kinetics after a soccer match. J Strength Cond Res. 2014;28(6):1517–23.
7. Ascensão A, Leite M, Rebelo AN, Magalhães S, Magalhães J. Effects of cold water immersion on the recovery of physical performance and muscle damage following a one-off soccer match. J Sports Sci. 2011;29(3):217–25.
8. Brownstein C, Dent J, Parker P, Hicks K, Howatson G, Goodall S, et al. Etiology and recovery of neuromuscular fatigue following competitive soccer match-play. Front Physiol. 2017;8:831.
9. Nédélec M, McCall A, Carling C, Legall F, Berthoin S, Dupont G. Recovery in Soccer: part ii-recovery. Sport Med. 2013;43(1):9–22.
10. Banfi G, Lombardi G, Colombini A, Melegati G. Whole-body cryotherapy in athletes. Sport Med. 2010; 40(6):509–17.
11. Banfi G, Melegati G. Effect on sport hemolysis of cold water leg immersion in athletes after training sessions. Lab Hematol. 2008;14(2):15–8.
12. Wozniak A, Wozniak B, Drewa G, Mila-Kierzenkowska C, Rakowski A. The effect of whole-body cryostimulation on lysosomal enzyme activity in kayakers during training. Eur J Appl Physiol. 2007;100(2):137–42.
13. Fonda B, Sarabon N. Effects of whole-body cryotherapy on recovery after hamstring damaging exercise: A crossover study. Scand J Med Sci Sport. 2013;23(5):e270–8.
14. Louis J, Theurot D, Filliard JR, Volondat M, Dugué B, Dupuy O. The use of whole-body cryotherapy: time-and dose-response investigation on circulating blood catecholamines and heart rate variability. Eur J Appl Physiol. 2020; 120:1733–43.
15. Selfe J, Alexander J, Costello JT, May K, Garratt N, Atkins S, et al. The effect of three different (-135oC) whole body cryotherapy exposure durations on elite rugby league players. PLoS One. 2014; 9(1):e86420.
16. Fonda B, De Nardi M, Sarabon N. Effects of whole-body cryotherapy duration on thermal and cardio-vascular response. J Therm Biol. 2014; 42:52–5.
17. Westerlund T, Smolander J, Uusitalo-Koskinen A, Mikkelsson M. The blood pressure responses to an acute and long-term whole-body cryotherapy (-110°C) in men and women. J Therm Biol. 2004;29(6):285–90.
18. Banfi G, Melegati G, Barassi A, Dogliotti G, Melzi d'Eril G, Dugué B, et al. Effects of whole-body cryotherapy on serum mediators of inflammation and serum muscle enzymes in athletes. J Therm Biol. 2009;34(2):55–9.
19. Hooper S, Mackinnon L, Howard A, Gordon R, Bachmann A. Markers for monitoring overtraining and recovery. Med Sci Sports Exerc. 1995;27:106–12.
20. Foster C, Florhaug J a, Franklin J, Gottschall L, Hrovatin L a, Parker S, et al. A new approach to monitoring exercise training. J Strength Cond Res. 2001; 15(1):109–15.
21. Duda RO, Hart PE, Stork DG. Pattern Classification. 2000. 13–29 p.
22. Field A. Discovering statistics using IBM SPSS statistics. Sage; 2013.
23. Morgans R, Orme P, Anderson L, Drust B, Morton JP. An Intensive Winter Fixture Schedule Induces a Transient Fall in Salivary IgA in English Premier League Soccer Players. Res Sport Med. 2014; 22(4):346–54.
24. Gleeson M. Mucosal immunity and respiratory illness in elite athletes. Int J Sports Med. 2000;21(Sup. 1):33–43.
25. Moreira A, Arsati F, Cury P, Franciscon C, de Oliveira P, de Araujo V. Salivary immunoglobulin a response to a match in top-level brazilian soccer players. J Strength Cond Res. 2009; 23(7):1968–73.