

Effects of selective head-and-neck cooling on brain injury-related biomarker levels and symptom rating following a boxing bout- protocol for an exploratory randomized trial

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Table of Contents

Original Manuscript..... 5

Supplementary Files..... 33

 Figures 34

 Figure 1..... 35

 Figure 2..... 36

 Figure 3..... 37

 Figure 4..... 38

CONSORT (or other) checklists..... 39

 CONSORT (or other) checklist 0..... 40

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Abstract

Background: Head impacts are common in contact sports such as boxing and occur at times of elevated core body and brain temperatures induced by the exercise. Following impact, elevated brain temperature may lead to the development of exacerbated brain injury that can be monitored by blood biomarkers.

Objective: We hypothesized that acute head-and-neck cooling, recently shown to shorten return-to-play in concussed ice hockey players, applied acutely following a boxing bout is associated with an attenuated concentration of blood biomarkers and improved symptom rating.

Methods: The trial is academically driven and funded by external and hospital research funds. Young, healthy elite boxers > 18 years old are recruited. Prior to, and immediately after a competitive boxing bout over 3x2 or 3x3 minutes, blood samples are drawn. Boxers are randomized to intervention or control management by 1:1 allocation prior to baseline testing. After the initial post-fight blood sample is drawn and symptom rating using the sports concussion assessment tool-5 (SCAT-5) has been collected, the boxers receive either acute selective head-and-neck cooling for 45 min, or routine post-fight management. The number of head impacts are counted in all boxers on match video recordings. In both groups, blood samples are drawn 45 minutes after the initial post-bout blood sample, as well as 3- and 6-days post-fight. At all time points for blood sampling, the number of symptoms (NOS) and symptom severity score (SSS) are assessed using the symptom rating part of the SCAT-5. The primary endpoint is the difference in biomarker levels (GFAP, NFL, tau, UCH-L1, neuronal-specific enolase) between immediately post-fight and pre-intervention levels, to those obtained at 6 days post-fight. The post-fight SCAT-5 NOS and SSS are secondary endpoints

Results: The primary endpoint is the difference in biomarker levels (GFAP, NF-L, tau, UCH-L1, neuronal-specific enolase) between immediately post-fight and pre-intervention levels, to those obtained at 6 days post-fight. The post-fight SCAT-5 NOS and SSS are secondary endpoints.

Conclusions: There is no treatment available for boxing-induced brain injury. Biomarkers are surrogate yet objective marker of brain injury, and the head-and-neck cooling treatment may attenuate the concentration of brain injury-related biomarkers as well as reduce symptoms induced by head impacts attained during a boxing fight. Potentially, head-and-neck cooling is a treatment option acutely following a boxing bout. Clinical Trial: ClinicalTrials.gov, ClinicalTrials.gov Identifier NCT06386484. Registered April 23, 2024.

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Original Manuscript

Effects of selective head-and-neck cooling on brain injury-related biomarker levels and symptom rating following a boxing bout-protocol for an exploratory randomized trial

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Key words: Sports concussion, biomarker, boxing, glial fibrillary acidic protein (GFAP), neurofilament-light (NFL),

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Abstract

Background

Head impacts are common in contact sports such as boxing and occur at times of elevated core body and brain temperatures induced by the exercise. Following impact, elevated brain temperature may lead to the development of exacerbated brain injury that can be monitored by blood biomarkers. Blood brain biomarkers S100B and GFAP reflect glial injury, NFL axonal injury, and NSE and tau neuronal injury. Time to peak levels post-injury for these biomarkers varies. Levels of S100B peak early post-injury while NSE, GFAP and tau are regarded as sub-acute markers, and NFL shows prolonged increases. Thereby we attempt to cover a large spectrum of first week post-fight brain blood biomarkers alterations, and their response to head-neck cooling.

Objective

We hypothesized that acute head-and-neck cooling, recently shown to shorten return-to-play in concussed ice hockey players, applied acutely following a boxing bout is associated with an attenuated concentration of blood biomarkers and improved symptom-rating.

Methods

The trial is academically driven and funded by external and hospital research funds. Young, healthy elite boxers ≥ 18 years old are recruited. Prior to, and immediately after a competitive boxing bout over 2-3x2 minutes, blood samples are drawn. Boxers are randomized to intervention or control management by 1:1 allocation prior to baseline testing. After the initial post-fight blood sample is drawn and symptom rating using the sports concussion assessment tool-5 (SCAT-5) has been collected, the boxers receive either acute selective head-and-neck cooling for 45 min, or routine post-fight management. The number of head impacts are counted in all boxers on match video recordings. In both groups, blood samples are drawn 45 minutes after the initial post-bout blood sample, as well as 3- and 6-days post-fight. At all time points for blood sampling, the number of symptoms (NOS) and symptom severity score (SSS) are assessed using the symptom rating part of the SCAT-5. The primary endpoint is the difference in biomarker levels (GFAP, NFL, tau, UCH-L1, neuronal-specific enolase) between immediately post-fight and pre-intervention levels, to those obtained at 6 days post-fight. The post-fight SCAT-5 NOS and SSS are secondary endpoints.

Results

Recruitment started November 2021 and is ongoing. So far, 41 boxers have been included: 20 controls and 21 cooled. Data collection started October 2024 following the completion of blood

sample analysis. We expect to recruit more boxers before summer 2025, but challenges with recruitment may limit this.

Conclusion

There is no treatment available for boxing-induced brain injury. Biomarkers are surrogate yet objective marker of brain injury, and the head-and-neck cooling treatment may attenuate the concentration of brain injury-related biomarkers as well as reduce symptoms induced by head impacts attained during a boxing fight

Trials registration

ClinicalTrials.gov, ClinicalTrials.gov Identifier NCT06386484. Registered April 23, 2024.

Introduction

Background and rationale

Trauma to the head is common in many contact sports, including boxing [1]. If the trauma to the head, or to the body with energy transmitted to the head, is sufficient to induce a range of clinical symptoms and signs that may or may not involve loss of consciousness, a sport-related concussion (SRC) has occurred [2]. In boxing, participants both receive and deliver, in average 32-40 blows to the head during a typical bout [3]. Those blows are referred as repetitive head impacts (RHI) [4, 5]. RHI differs from the definition of sport related concussion (SRC) by the absence of the acute symptoms typically observed following an SRC. However, similar to after several SRCs cumulative effects of RHI include early-phase changes in brain function and increased risk for neurodegenerative disorders at long-term. Neuropathological consequences of RHIs in boxing have been comprehensively investigated in previous studies, illustrating structural alterations to the brain [2, 6]. More specifically RHI may lead to, *e.g.*, cognitive impairment, mood disorders, and motor control problems [7-9].

Several studies have shown that retired athletes who have sustained multiple RHI and SRCs during their career may develop chronic traumatic encephalopathy (CTE); a progressive neurodegenerative disease associated with memory loss, depression, personality changes, and dementia [1, 8, 10-13]. At present, CTE can only be diagnosed *post-mortem* where key findings include irregular aggregation of

phosphorylated tau protein at the depths of cortical sulci [8].

In sports, the strenuous exercise leads to elevated core body- and brain- temperature [14], which may exacerbate the brain injury induced by head impacts. Recently, we showed that selective head-and-neck cooling for 45 minutes, aiming to rapidly normalize the elevated brain temperature, applied acutely post-SRC in elite ice hockey players resulted in earlier return-to-play and a smaller proportion of players with prolonged symptoms following the SRC [15]. However, there is a lack of objective outcome measures for assessing the effects of head-and-neck cooling in athletes.

There is a growing body of literature on the use of biomarkers of brain injury in athletes. In Olympic boxers, levels of cerebrospinal fluid biomarkers such as S100 calcium-binding protein (S100B), Tubulin-associated unit (Tau), Neurofilament light (NFL) and Glial fibrillary acid protein (GFAP) were collected at baseline, 1-6 days post-fight and after 14 days after RHI attained in a boxing bout [16, 17]. Additionally, the biomarkers showed both acute and cumulative effect of the impacts, with a lack of normalization of NFL and GFAP after the rest period, which may indicate an ongoing injury process [16, 17]. In addition, inflammatory biomarkers such as tumor necrosis factor alpha and Interleukin 6 and muscle injury biomarkers (alanine aminotransferase (ALT), aspartate aminotransferase (AST) and creatinine increased in male elite boxers following a fight [18].

When boxing and mixed martial art (MMA) were compared, retired boxers had a higher plasma GFAP levels, and active boxers higher plasma NFL than MMA fighters [19]. In addition, serum NFL levels were elevated both at 7-10 days post bout and after 3 months of rest in boxers compared to controls. This study also observed a significant increase in NFL at 7-10 days post bout comparing levels obtained after a high number of head impacts (more than 15 hits to the head or experienced grogginess during or after bout) to those obtained after fewer head impacts (fewer than 15 head hits) [20]. These studies indicate that selected biomarkers may remain elevated during the 1st post-injury

week. In this randomized trial, we hypothesized that biomarkers could serve as objective marker for the efficacy of selective head-and-neck cooling on attenuating brain injury in elite boxers, assessed by blood biomarkers immediately prior to and during the first 6 days after a competitive boxing fight.



Objectives

Research hypothesis: Acute and selective head-and-neck cooling immediately after a boxing bout attenuated brain injury as evident by a reduced concentration of brain injury-related biomarker levels during the 1st post-fight week.

Study objectives.

Primary objective: To determine whether selective head-and-neck cooling attenuates the concentration of brain injury biomarkers.

Secondary objectives:

- To determine whether selective head-and-neck cooling improves symptom rating using the Sports Concussion Assessment tool- 5 (SCAT-5).
- To determine whether any biomarker increase post-fight, compared to baseline levels, is associated with the number of head impacts
 - To determine any adverse event associated with the head-and-neck cooling

Methods: participants, interventions, and outcomes

Study setting

The study will take part predominately in southern parts of Sweden due to logistic reasons, aiming for feasibility of follow up with biomarkers at distinct post-fight time points. Blood samples will be collected at the site of the competitive boxing tournament or at the local boxing clubs and transferred to the biobank at Lund University Hospital. All data will be collected in Sweden.

The trial is academically driven by local funding. The trial will be conducted at Lund University, Lund, Sweden.

Trial design

The head-and-neck Cooling of Brain temperature In BOXing (COBIBOX) trial is designed as a randomized, controlled, open, single-center superiority trial with two parallel groups and a primary

endpoint of biomarker changes during the first 6 post-fight days.

Clinical secondary outcome assessments of number of symptoms (NOS) and symptom severity score (SSS) using the symptom rating part of the of the SCAT-5 during the initial six post-injury days.

Randomization will be performed in blocks with 1:1 allocation.

Eligibility criteria

Inclusion criteria:

1. Male and female elite boxers < 40 years old and ≥ 18 years old.
2. Cleared for participation by the medical staff of the Swedish Boxing Association prior to boxing bout.
3. Informed consent by each participant prior to the competitive event

Exclusion criteria:

1. > 40 years old or < 18 years old
2. Participation in competitive fights, match-sparring or any training causing additional head impacts during the six days post-fight follow up period after being included in the study.
3. History of autoimmune neurological diseases or a neurodegenerative disorder.
4. History of previous traumatic brain injury resulting in an intracranial haemorrhage.

Procedures and measurements

All boxing clubs are thoroughly informed of the protocol prior to study initiation and prior to each tournament. Boxers between the ages of 18 and 40 are included. At each tournament, allocation to the treatment takes place the first morning and the boxers are randomised by 1:1 by a randomly drawn paper slip with a sign if the boxer will receive either selective head-and-neck cooling or routine post-fight medical surveillance by an on-site physician. The selective head-and-neck cooling is to be initiated within 10 minutes post-fight with a duration of a minimum 45 minutes.

After the boxer completes the fight (Figure 1-2), he or she follows the researcher to the cooling station and continues the head-and-neck cooling protocol. In some boxing events, a boxer may participate in two bouts if they win their first bout (Figure 2). The next day, during the finals, they will undergo the same protocol as on their first day, including SCAT5 assessments and blood sample collection [21]. As mentioned in Figure 2 the start of intervention or return to play protocol starts after their last fight.

The PolarCap System consists of a portable cooling system, designed to reduce brain temperature by controlled cooling of the scalp and neck with a circulating coolant (PolarCap Coolant, PolarCool AB, Lund, Sweden). The coolant is maintained at 0°C and flows through a silicone-based head cap. An insulating neoprene cover is put on top of the cap for isolation. The players are allowed to relax (sitting or supine) as long as the cooling head cap and neoprene cover remained in the place on the head [22] Figure 3. Should the boxer experience discomfort and wish to discontinue the intervention, the cap is removed and the athlete excluded from the study. All boxers follow return to fight protocol. The standardized protocol is return to physical exercise during the first 6 post-fight days. During this time, the boxer may do all forms of physical exercise except sparring, match fight or other activities resulting in impacts to the head or body.

Blood samples are collected at the following time points for all participants: The morning of the first boxing fight at day 1 (baseline, at rest), immediately after the fight, 45 minutes after the fight in, and after 3 and 6 days in all boxers. The blood samples obtained at 3- and 6-days post-fight will be drawn by a study nurse or a researcher (AaH) at the participant's boxing club. The samples are transported to the hospital in a mobile refrigerator to maintain a temperature of 5-8° C. Thereafter the serum samples are centrifuged at 3000 RPM, at 4° C for 10 minutes, after which 800 -1200 µL is transferred using a pipet to 2ml tubes and stored at -80° C.

At each time point for blood sample collection, boxers are also administered the SCAT-5

questionnaire, which is a self-report questionnaire with information on 22 different symptoms, each with a severity grading of 0-6, where 0 indicates no symptoms and 5-6 indicates severe symptoms. The maximum total score is 132. All boxing bouts are video recorded for later analysis. The total hits per bout, strikes to the head, strikes to the body and visible evidence of neurological disturbance (loss of consciousness, impaired balance and/or coordination, vacant expression *etc.*) will be assessed. After completion of the study, the boxers will be interviewed by phone to answer questions about their previous boxing, knock-out and concussion history.

Participant timeline

	STUDY PERIOD							
	Enrolment	Pre-allocation	Allocation	Post-allocation				Close-out
TIMEPOINT	-t ₁	Baseline	0	Post-fight	45 min post-fight	Day 3	Day 6	1 week post-fight
ENROLMENT:	X							
Eligibility screen	X							
Informed consent	X							
Basic Medical examination	X							
Allocation			X					
INTERVENTION S:								
Selective head and neck cooling (A)				X				
Return to fight protocol (A+B)				X	X	X	X	
Collecting blood sample		X		X	X	X	X	
ASSESSMENTS (SCAT5):								
SCAT5		X						
SCAT5 - follow up				X	X	X	X	
Boxing history								X

A = intervention group.

B = control group.

Sport concussion assessment Tool 5 (SCAT5)

Ethics

The study outlined in this article will be carried out in accordance with the Declaration of Helsinki and obtained regional ethics committee approval in Lund, Sweden (decision number Dnr 2022/06195). Prior to their enrolment in the trial, all study participants will provide written informed

consent. Each participant signs the form, containing comprehensive study details.

Approval from the Swedish Boxing Association to include amateur and professional boxers registered in the Swedish Boxing Association (SBA) in the study will be received before the study is initiated. Forms describing the study are sent to all boxing clubs in southern Sweden that would participate in the tournaments and have enrolled boxer over 18 years old. All participants are informed of their rights to terminate their participation in the study at any given time without need to provide a reason for doing so.

Informed consent

The informed consent consists of ten detailed points. It begins with a **request for participation that** describes what the boxers will be involved in. The **background and aim** section explain the purpose of the study and the facts supporting it. The **study procedure** outlines the collection of SCAT5 questionnaires, blood samples, and the possibility of cooling. **Biomarker collection** is covered by the biobank policies and includes the category of biomarkers that will be analyzed. The **risk** section highlights any potential risks associated with participation. **Handling of personal data** explains data protection measures in accordance with GDPR, while **data management and privacy** further details how data is stored and covered under GDPR regulations. The **insurance** section clarifies the responsible parts that insures the participants during the study. **Voluntary participation** ensures that participants are informed of their right to opt out at any time, even after inclusion. Lastly, the **responsible staff** section provides information about the staff in charge and their contact details.

Ethical consideration

All questionnaires and data collection methods were reviewed and approved by the Swedish Ethical Review Authority in Uppsala, Sweden. The decision number for the original application is Dnr. 2019-05463. The submitted protocol to the Swedish Ethical Review Authority included detailed

information on the intervention, data confidentiality, blood collection, blood sample storage, and measures to ensure the protection of participants' privacy. A study protocol was provided with the ethical application. All participants' data are anonymized from the time of inclusion, prior to allocation, by assigning a code containing letters and numbers. This code will be attached to the participants' blood samples and SCAT5 score files. Each participant will receive a compensation of 1,000 SEK for their time, inconvenience, and any potential discomfort associated with participation. The consent information emphasizes the voluntary nature of participation, including the possibility of opting out at any stage. It also specifies who will obtain informed consent from the participants. Specific contact information for the research team is provided to participants for any questions or concerns.

Study population

Fourteen boxing clubs whose fighters enrolled in 5-7 boxing events in southern Sweden will be contacted and informed about the study and its inclusion criteria.

Allocation

After all boxers approve participation in the study during the tournament, and sign the informed consent, the study staff makes allocation on paper slips that equals the numbers of participants. Then the boxers randomly, by drawing a paper slip, receives either a sign for receiving head-and-neck cooling (C= cooling) or NC (no cooling) (Fig1-2). All allocations are finalized a minimum of 3 hours prior to the start of the tournaments.

The enrolment of participants will be done by a researcher (AaH) aided by boxing coaches.

After the boxing bout, and after the boxer is evaluated for red flags mandating transport to nearest hospital, the boxer is brought to the intervention station to apply the Polarcap® system as previously described.

Who will take informed consent?

Informed consent documents will be sent for approval to the Swedish Boxing Association, after which it will be distributed to each club. A responsible researcher/physician (AaH) will inform the participants verbally and in written form either days before the competition or on the same day. Each participant will be informed about the study, informed of the possibility to withdraw from participation at any time, and provided time to ask questions. Prior to randomisation, each participants signs the informed consent form.

Biomarker analysis

Venous blood is collected in 3.5 ml serum and EDTA plasma tubes generated at room temperature and stored in a mobile refrigerator at 4-8°C until centrifuged at 4 °C for 10 minutes at 3000 rpm. Remaining supernatant is transferred to 2 ml cryotubes and stored at –80°C until analyzed. Once the study has been completed, blood biomarkers for brain injury (S100B, GFAP, tau forms, NFL and NSE) will be measured in coded samples using established Single molecule array (for GFAP, tau and NFL) by Quanterix (Billerica, MA) and electrochemiluminescence immunoassays (for S100B and NSE) by Roche Diagnostics (Basel, Switzerland) immunoassays [23] in the Clinical Neurochemistry Laboratory by board-certified laboratory technicians who will be blinded to the intervention.

Data collection

The Sport Concussion Assessment Tool (SCAT5) form will be filled out at time of biomarker analysis and used as a secondary endpoint of the study. The boxing bouts take place either in events consisting of single bouts for each boxer or in tournaments where two bouts are possible (semi-final and final). For boxers participating in two bouts at the same event the baseline, post-fight, and 45-minute post-fight scores and biomarker test will be re-taken. Values related to the second fight, will

be considered in the analysis. The analysis of symptom severity scores obtained by SCAT5 at the set timeline will facilitate the interpretation of outcome between the boxers independent of their previous boxing history after the completed 6-day follow up. Boxing history will be obtained by phone call interview at the 6-day follow-up or onsite. The follow up for day 3 and 6 will be organized by the local clubs prior to the 1st post-match training session, or at the study hospital.

Sample size and statistical power

Based on previous reports, an approximately 10% increase in key biomarkers is observed after a concussion in athletes [16]. We hypothesized a 20% reduction of biomarker level by acute head-and-neck cooling. We assumed equal variance of the cooled and control groups. The selected power was

set to 0.8. Power analysis was conducted using the following formula:
$$n = \frac{2 \cdot (Z_{power} + Z_{alpha})^2 \cdot S D^2}{effect\ size^2}$$
 [24].

Based on the power calculations, a total number of 20 participants per group is expected to yield a sufficient statistical power. Since dropouts and missed sample times can be anticipated we aim for group sizes of 25 per group.

Recruitment

Participants are recruited by one researcher (AaH) by sending information about the study to the Swedish Boxing Association that approves participation, in written form or verbally. Thereafter the study must be approved by the organisers of the boxing tournament. Finally, recruitment of boxers who fulfil inclusion criteria and sign the written consent form after verbal and written information about the study is provided.

Adverse events

Any adverse events reported by the participant during the study period is noted and reported.

Specifically, any deviation from protocol (*e.g.*, sample time point, duration of head-and-neck cooling) will be noted. Symptoms, such as headache, nausea and dizziness, are reported to the medical doctor and study researcher (AaH), although these symptoms may also be caused by the SRC *per se* [15]. No adverse events are expected, based on our previous study where 61 elite ice hockey players were cooled without any reported adverse events [15].

Results

The study flow is presented in Figure 1-2. The study began in November 2021 and is expected to be completed before the summer of 2025 Figure 4. Based on our sample size calculations, we aim to recruit approximately 40-50 participants. The first results are anticipated to be submitted for publication in the autumn of 2025. This study evaluates the effectiveness of selective head and neck cooling in reducing blood-brain biomarkers and SCAT5 scores during the first week after boxing

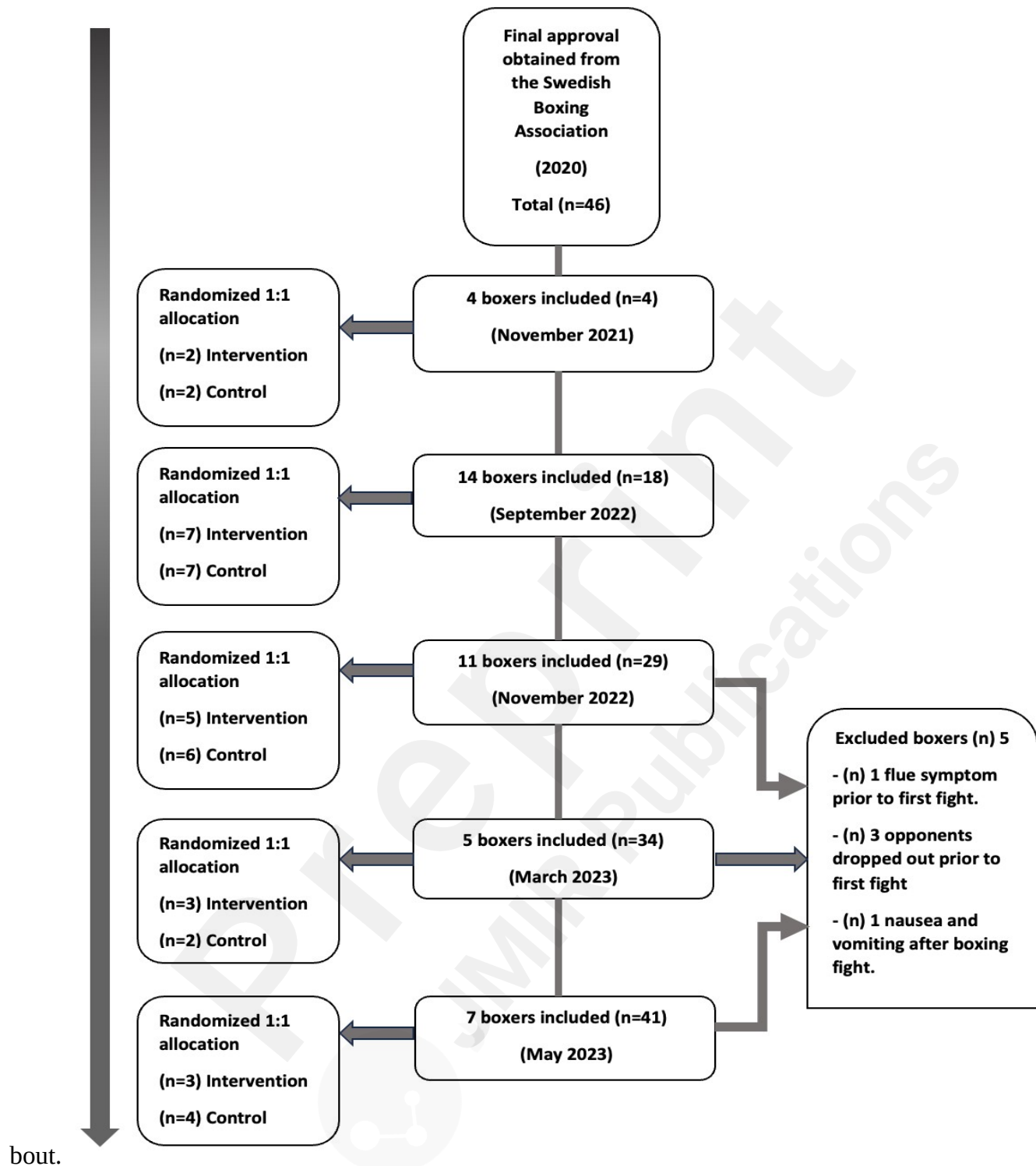


Fig 4. All allocation and randomization occur after signed informed consent and before baseline testing. The three excluded boxers, whose opponents dropped out, went through baseline SCAT5 testing and blood sample collection. The participant that experienced nausea and vomiting chose to withdraw from participation in the study after his boxing bout prior to any further testing.

DISCUSSION

During a boxing fight, boxers typically attain a high number of blows to the head, which may lead to brain injuries. Due to the strenuous activity, these blows occur at a time of elevated core body temperature, which may exacerbate the brain injury. To date, there are no approved treatments for boxing-induced brain injuries. A blow to the head may lead to elevation of blood biomarkers concentrations that reflect the degree of the brain injury caused by the impact. There is an increasing interest of these biomarkers that include markers for large caliber axon injury (NFL), neuronal injury (NSE, tau) and glial injury (S100B, GFAP). Our present study may show whether the level of biomarkers is related to the number of hits or concussions received, the symptom development after the fight and most importantly the effects of the cooling intervention.

Head-and-neck cooling was shown to decrease brain temperature measured by magnetic resonance imaging (MRI) [25], and improved cognitive outcome when applied acutely post-concussion [26]. In a recent clinical trial including 132 concussed ice hockey players, a shorter recovery time was observed after acute selective head-and-neck cooling for at least 45 minutes [15]. A shorter recovery was also observed when cooling was initiated up to 8 days post-concussion [27]. To provide an objective outcome measure the cooling intervention in the present study, the primary outcome is blood biomarker levels, of which GFAP, NFL, S100B and tau may be the most important. The chosen timepoints for collecting blood-brain biomarkers aim to assess the early-phase biomarker response to boxing matches, with subsequent evaluation of the head and neck cooling effect and its potential early impact. S100B is associated with an acute astrocytic response, which typically peaks within hours, followed by GFAP with a peak of 24h [28]. This makes post-fight day 3 a valuable timepoint to capture a delayed peak or sustained elevation of the selected biomarkers. While NFL levels may remain elevated at longer time points post-fight, we had to use 6 days as our latest evaluation time point. This is based on the guidelines of the Swedish Boxing Association, which

prohibit boxers from competing or having fights within 6 days of their most recent tournament. Thus, many boxers are assigned to match sparring or other tournaments after 6 days.

The selection of biomarkers in the present trial is based on several previous studies [29, [30], mainly in traumatic brain injury (TBI) and neurodegenerative disorders [19]. Currently, both tau isoforms and GFAP is used in prediction and diagnosis of neurodegenerative diseases, particularly Alzheimer's disease, and S100B in mild traumatic brain injury guidelines [31-33].

In numerous reports on mild, moderate or severe traumatic brain injury, blood and CSF biomarkers have been used in different time points post-injury [29]. Following boxing bouts, tau, NFL, GFAP, and S100B are all increased in the early phase after boxing. Of these, NFL, GAP and tau are increased several weeks- to months post-fight [16, 17, 34]. In addition,

Strenuous exercise has been reported to increase biomarkers such as S100B [35, 36]), while leading to a reduction in GFAP and no alteration in NF-L levels [37]. When comparing strenuous exercise to contact sports such as boxing, boxers showed a significant increase is notable in biomarker levels, particularly in GFAP and NFL [17].

Blood S100B levels were significantly elevated in both competitive boxers and high levels exercised athletes such as runners [16, 38]. These findings argue that boxing causes the elevation of concentration of several biomarkers reflecting the brain injury, and be associated with lead to long-term neurological consequences.

The role of brain temperature in acute brain injury has been evaluated in numerous trials. In particular, elevated brain temperature may negatively impact functional and neurological outcomes following traumatic brain injury [39, 40]. While systemic hypothermia has not been associated with improved outcomes in severe traumatic brain injury, we hypothesized that controlling body temperature may be an important factor in the recovery of boxing-induced brain injury. The primary aim of the head-and-neck cooling is not to provide cerebral hypothermia, it is to attenuate exercise-

induced hyperthermia and to rapidly normalise the elevated brain temperature.

During exercise, elevated temperature is associated with a higher demand of oxygen and energy metabolism in the brain [41, 42], resulting in exacerbation of the injury mechanism initiated by head impacts during boxing. Our hypothesis is that brain cooling can rapidly normalize brain temperature, thereby reducing metabolic demand resulting in attenuated secondary injury and leading to beneficial effects for the recovery phase after a fight.

Should this explorative study provide evidence of beneficial effects by head-neck cooling, a multicenter study (preferably with later post-fight time points) is warranted.

Strengths and limitations

The present study has the potential to objectively evaluate the effect of head- and neck cooling on blood biomarker levels. Moreover, it can provide unique results on biomarker concentration changes following repeated head impacts at several time points post-fight.

Outliers in biomarker concentrations are expected, although since each boxer will be his/her control, percentage changes from baseline can be calculated. A 20% reduction of biomarker concentrations is reasonable based on the rapid application of the head- and neck cooling. The potential placebo effect of cooling on symptom ratings is also taken into consideration, by using objective biomarker concentrations, highlighting the necessity of biomarker assessment to evaluate cooling's physiological impact.

Conclusion

This study is structured to evaluate early-phase objective and subjective changes in boxers during the first week post-fight, and to study the effects of head- and neck cooling on these changes. In conclusion, the present trial may provide objective biomarker evidence for the role of attenuating cerebral hyperthermia by selective head-and-neck cooling acutely following a boxing bout.

Trial status

Recruitment started on November 4, 2021. Approximate completion of final recruitment is expected in the first weeks of January 2025.

Protocol version 1.0, 2024-04-01.

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Marit Bäckström for administrative assistance.

The results of this trial will be presented in an appropriate scientific journal after the trial is completed.

Authors' contributions

All authors have reviewed, edited, and approved the manuscripts final form. AaH and NM wrote the original draft. AaH, YT, HZ, KB and NM were responsible for conceptualization of the trial.

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Availability of data and materials

Data generated from this trial may be received from the authors upon reasonable request.

Declarations**Ethics approval and consent to participate.**

See ethics part.

Consent for publication.

When the participant/boxer is informed of the trial, information is given about the intent of publishing the results, and if consent is given for participation in the trial, consent for publications is given as well.

Competing interests

NM and YT are scientific advisors for PolarCool Inc. Lund, Sweden. Polar Cool provides the cooling helmet, although has not influenced the design of the study protocol. HZ has served at scientific advisory boards and/or as a consultant for Abbvie, Acumen, Alector, Alzinova, ALZPath, Amylyx, Annexon, Apellis, Artery Therapeutics, AZTherapies, Cognito Therapeutics, CogRx, Denali, Eisai, Merry Life, Nervgen, Novo Nordisk, Optoceutics, Passage Bio, Pinteon Therapeutics, Prothena, Red Abbey Labs, reMYND, Roche, Samumed, Siemens Healthineers, Triplet Therapeutics, and Wave, has given lectures in symposia sponsored by Alzecure, Biogen, Cellectricon, Fujirebio, Lilly, Novo Nordisk, and Roche, and is a co-founder of Brain Biomarker Solutions in Gothenburg AB (BBS), which is a part of the GU Ventures Incubator Program (outside submitted work). KB has served as a consultant, at advisory boards, or at data monitoring committees for Abcam, Axon, Biogen, JOMDD/Shimadzu. Julius Clinical, Lilly, MagQu, Novartis, Prothena, Roche Diagnostics, and Siemens Healthineers, and is a co-founder of Brain Biomarker Solutions in Gothenburg AB (BBS), which is a part of the GU Ventures Incubator Program. IR has served on advisory boards and given talks sponsored by Biogen, Bayer, Merck, Roche, GlaxoSmithKlein, TEVA, Sanofi and UCB.

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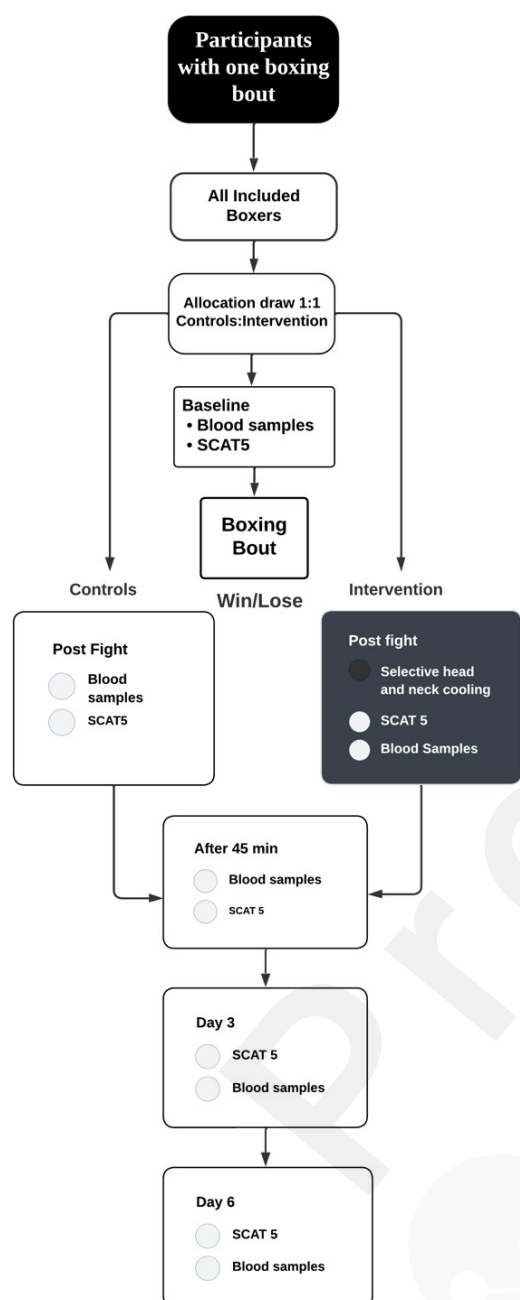
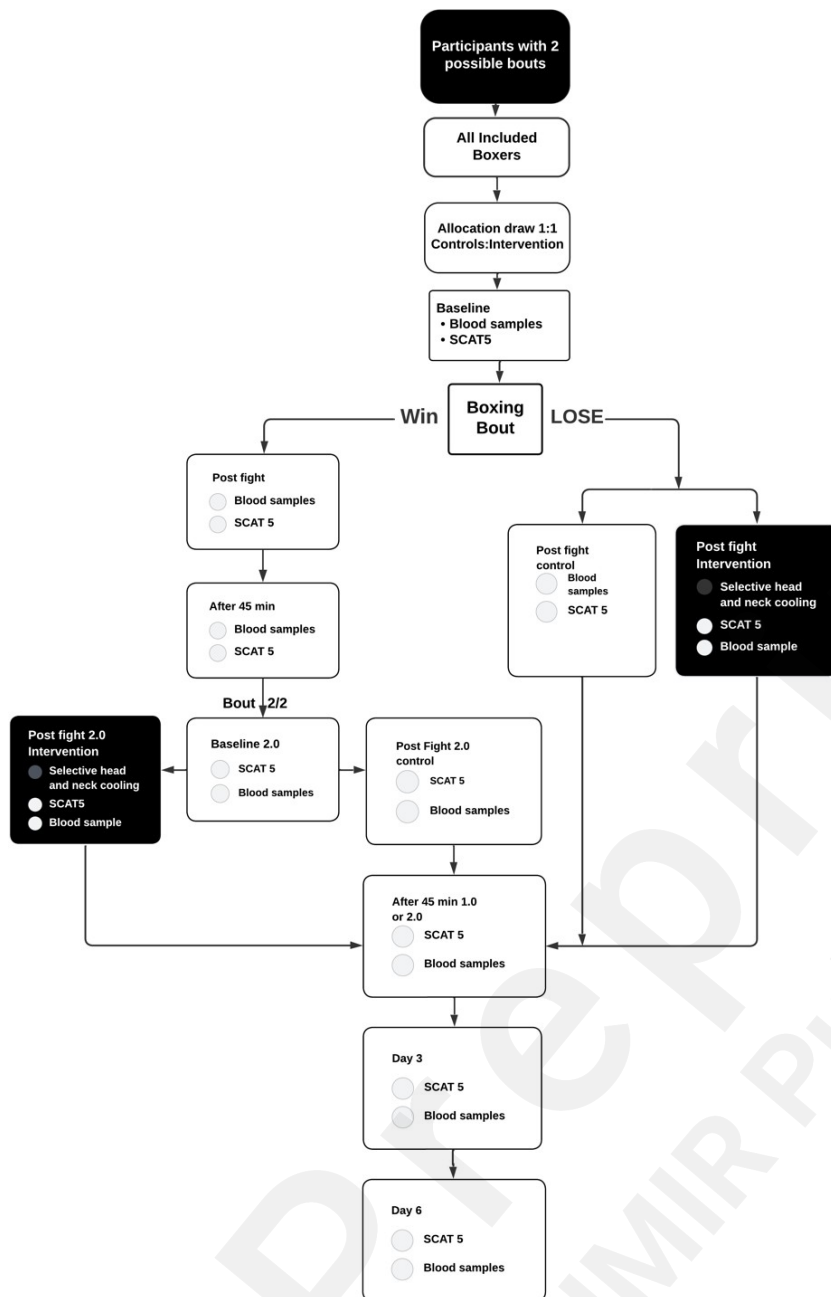


Fig.1

Boxers that participate in direct final bouts or single-fight events will only engage in one boxing bout. Participants will be randomly allocated to either the control or intervention group, involving selective head-and-neck cooling. Baseline SCAT5 and blood samples will be collected. Subsequently, post-fight SCAT5 and blood samples will be collected for both groups. Additionally, the intervention group will undergo selective head-and-neck cooling for 45 minutes. Following this, both the control and intervention groups will follow the same protocol, undergoing SCAT5 and blood sample collection at 45 minutes post-fight, as well as on Day 3 and Day 6 for follow-up.

**Fig.2**

Boxers who participate in tournaments can either fight one or two boxing bouts, depends on whether they win or lose. The selective head-and-neck cooling take part after last boxing bout (dark coloured boxes). For boxer who fights bout 2/2, new baseline SCAT 5 and blood samples will be collected, shown as baseline 2.0 in the figure.

**Fig.3**

A) The PolarCap[®] unit is a powerful mobile cooling system.

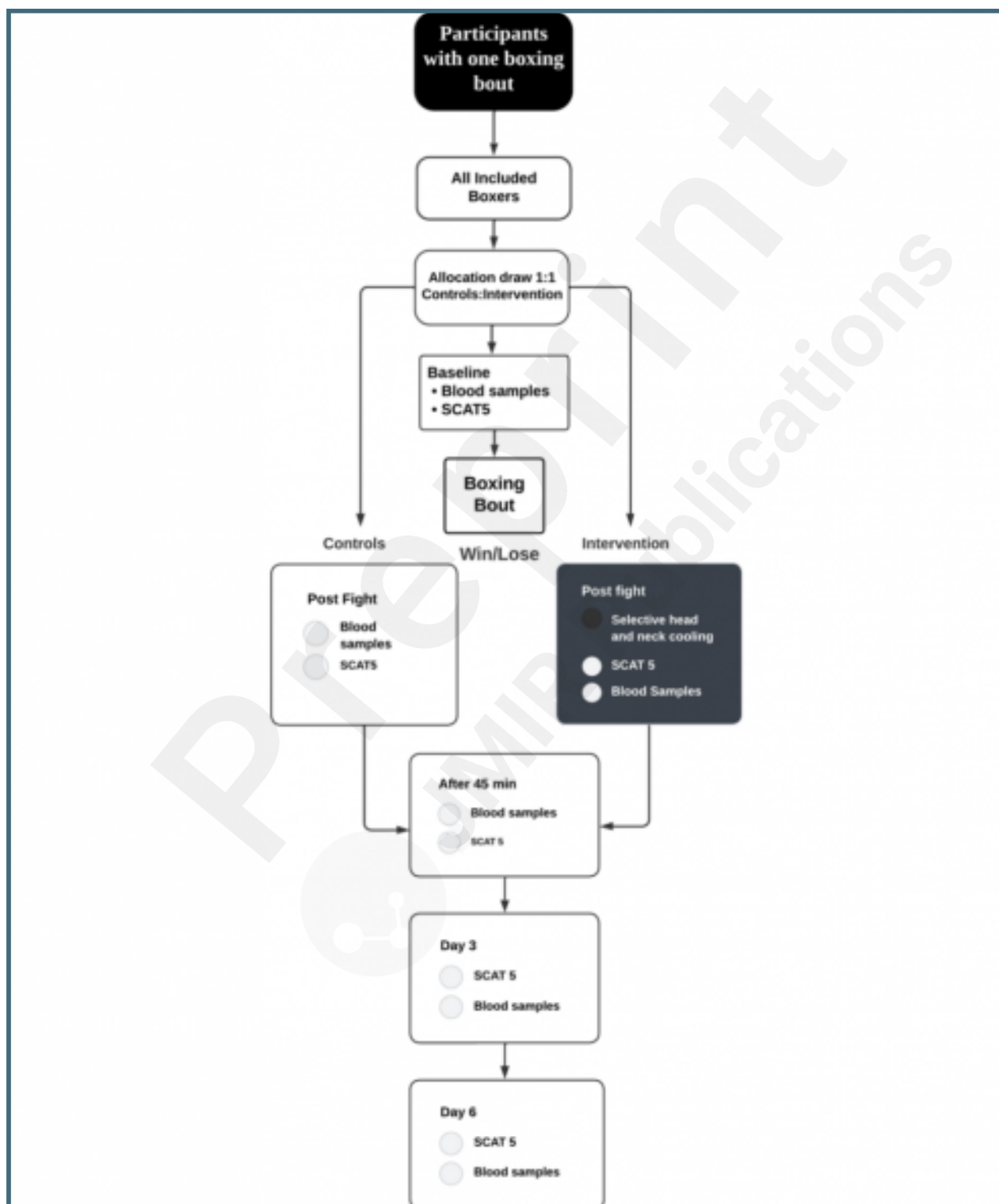
B) The Polar Coolant is transported through the silicone head cap.

C) To insulate the cold, neoprene cover is applied on top of the cap.

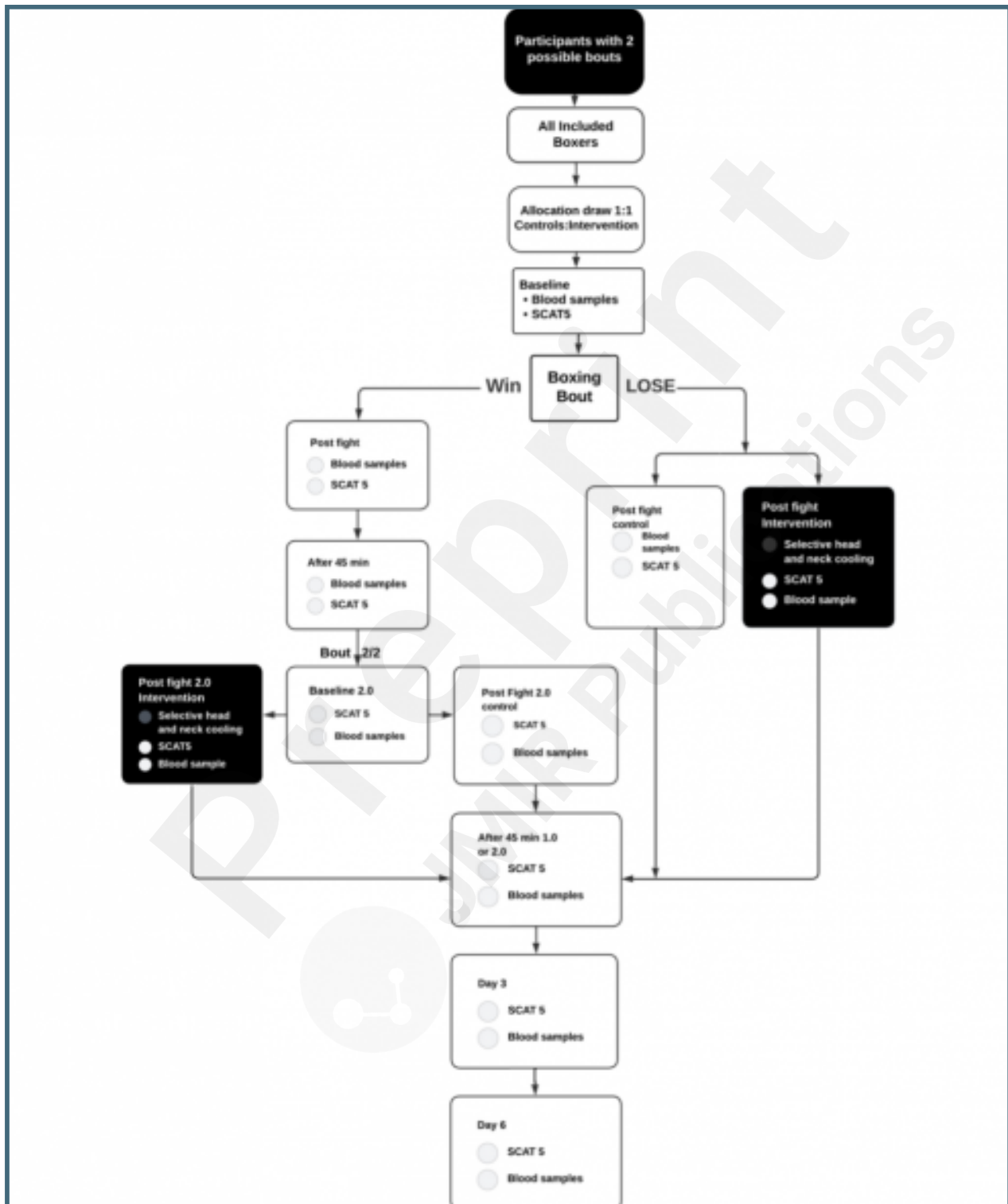
Supplementary Files

Figures

Boxers that participate in direct final bouts or single-fight events will only engage in one boxing bout. Participants will be randomly allocated to either the control or intervention group, involving selective head-and-neck cooling. Baseline SCAT5 and blood samples will be collected. Subsequently, post-fight SCAT5 and blood samples will be collected for both groups. Additionally, the intervention group will undergo selective head-and-neck cooling for 45 minutes. Following this, both the control and intervention groups will follow the same protocol, undergoing SCAT5 and blood sample collection at 45 minutes post-fight, as well as on Day 3 and Day 6 for follow-up.



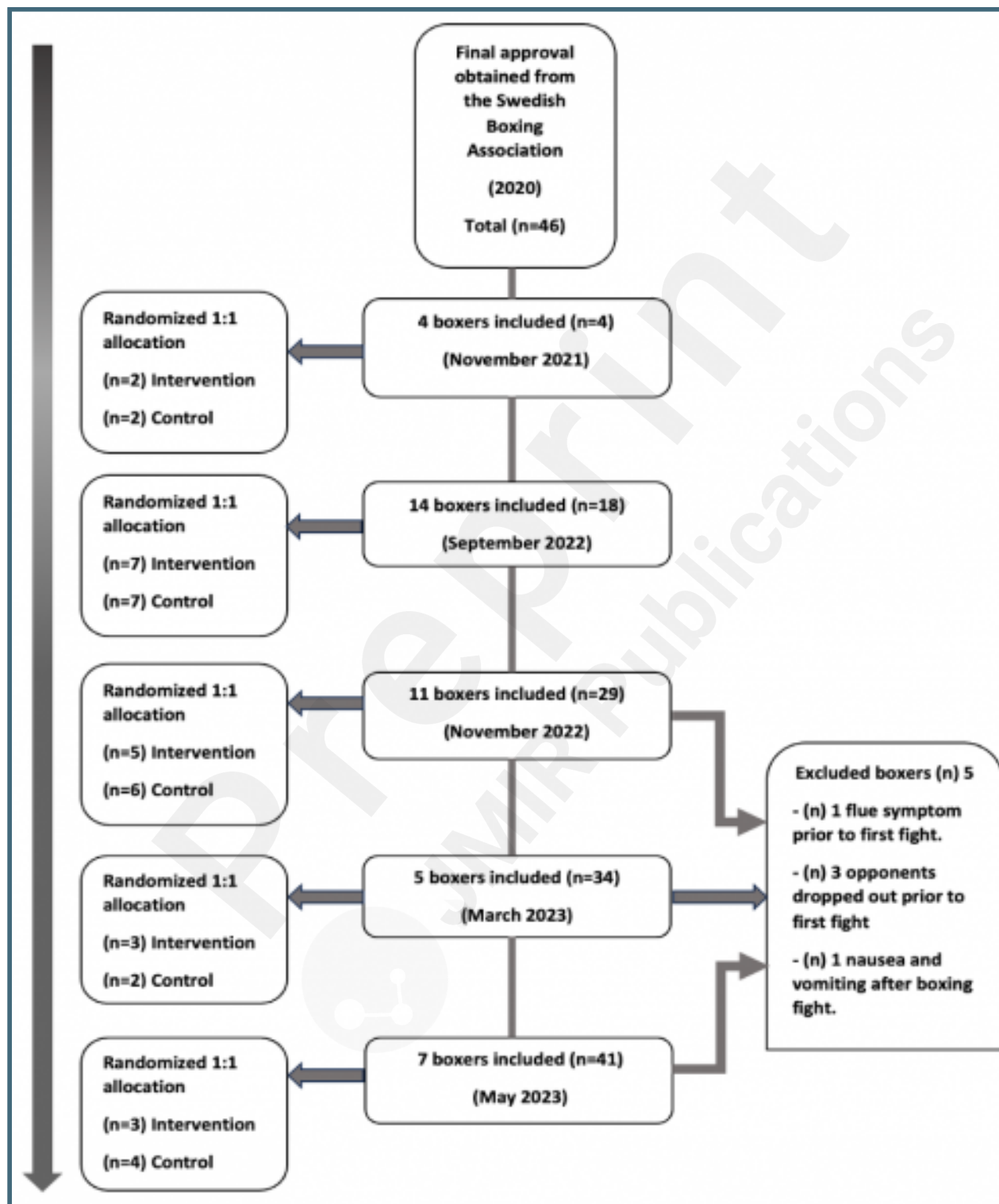
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A) The PolarCap® unit is a powerful mobile cooling system. B) The Polar Coolant is transported through the silicone head cap. C) To insulate the cold, neoprene cover is applied on top of the cap.



Fig 4. All allocation and randomization occur after signed informed consent and before baseline testing. The three excluded boxers, whose opponents dropped out, went through baseline SCAT5 testing and blood sample collection. The participant that experienced nausea and vomiting chose to withdraw from participation in the study after his boxing bout prior to any further testing.



CONSORT (or other) checklists

SPIRIT CHECKLIST.

URL: <http://asset.jmir.pub/assets/677f4086ffbf88a4d27627b92ed508a.pdf>

