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The effects of total sleep deprivation and ammonia inhalants on the cognitive and physical abilities of military personnel

Dissertation

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I declare that I complied with this dissertation under my supervisor's leadership and affirm that this dissertation is a representation of my work. It has not been previously included in a dissertation submitted to this institution or any other for a degree or other qualifications.

Prague, June 2023 Signature ……………………

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Abstract

Given that this dissertation is built up upon three sequential studies, the objectives, methods and results of the thesis are accordingly divided into three distinct subsections (a, b, and c), each corresponding to an individual study.

Title:

The effects of total sleep deprivation and ammonia inhalants on the cognitive and physical abilities of military personnel

Objectives:

a) Conducting a literature review to identify the current understanding on the topic and substantial gaps in the literature by investigating the impact of ammonia inhalations on cognitive and physical performance.
b) Conducting a methodological study to examine lacking test-retest reliability of standard-issued static and dynamic military shooting protocols for comprehensive investigation of the of total sleep deprivation impact and ammonia inhalants on handgun shooting accuracy.
c) Conducting a control trial study to investigate the effectiveness of ammonia inhalants in altering the impact of 36 hours of total sleep deprivation on battery of cognitive and physical performance tests relevant for military personnel.

Methods:

a) For the purpose of this literature review, a search of the following electronic databases by the author JM was performed: MEDLINE by PubMed, SCOPUS, Web of Science, and Google Scholar. Due to the paucity of research on the topic, search terms were quite broad and included a variety of terms including: ammonia, inhalation, performance, and smelling salts. Additionally, due to the lack of peer-reviewed articles, a manual search from the reference list of all retained articles was performed until April 2020.
b) Twenty soldiers (22 ± 1.9 yrs; 81.7 ± 8.6 kg; 184.9 ± 5.9 cm) participated in two static and dynamic shooting protocols trials on three separate days to determine the between-day and same-day test-retest shooting accuracy reliability. The accuracy (total points scored) for each trial was recorded and analysed for 1) the reliability of each shooting protocol with two-way mixed effects intra-class

correlation (ICC) with a coefficient of variation (CV) and the standard error of measurement (SEM) and 2) differences in shooting accuracy points scored between days and trials by two-way RM ANOVA.

c) Eighteen male cadets (24.1 ± 3.0 yrs; 79.3 ± 8.3 kg) performed 5 identical testing sessions during 36 hours of total sleep deprivation (TSD) (after 0 [0], 12 [-12], 24 [-24], and 36 [-36] hours of TSD), and after 8 [+8] hours of recovery sleep. During each testing session, the following assessments were conducted: Epworth sleepiness scale (ESS), simple reaction time (SRT), shooting accuracy (SA), rifle disassembling and reassembling (DAS), and countermovement jump height (JH). Heart rate (HR) was continuously monitored during the SA task, and a rating of perceived exertion (RPE) was obtained during the JH task. At each time point, tests were performed twice, either with AI or without AI (CON), in a counterbalanced order.

Results:

a) To date, there is a lack of evidence to support anecdotal claims of increased cognitive arousal and greater strength performance. However, there may be a short-term effect of ammonia inhalants on the cardiorespiratory system (possibly increasing breathing rate and heart rate approximately 15 to 30 seconds), but further research is needed to support these findings and to determine how the short-term cardiorespiratory effects may affect other physiological and performance measures. Lastly, although evidence does not indicate that ammonia inhalants are dangerous in healthy populations, sport and health professionals should be aware of the potential risks of AIs to prevent any unlikely, but possible, difficulties.

b) The results indicated good between-day test-retest reliability of the average of two trials of both the static (ICC = 0.837 [0.659, 0.930], CV = 3.78%, SEM = 3.37) and dynamic (ICC = 0.806 [0.597, 0.917], CV = 4.73%, SEM = 3.73) protocols. Additionally, there was moderate between-day test-retest reliability of a single trial for static (ICC = 0.703 [0.383, 0.872], CV = 3.47%, SEM = 3.11) and dynamic (ICC = 0.585 [0.219, 0.810], CV = 4.17%, SEM = 3.30) protocols, and moderate same-day test-retest reliability for static (ICC = 0.510 [0.248, 0.741], CV = 2.57%, SEM = 2.31) and dynamic (ICC = 0.510 [0.243, 0.742], CV = 4.30%, SEM = 3.39) protocols across the last two trials.

c) There was no condition × time interaction in any test, but there was faster SRT (1.6%; $p = 0.007$) without increasing the number of errors, higher JH (1.5%; $p = 0.005$), lower RPE (9.4%; $p < 0.001$), and higher HR (5.0%; $p < 0.001$) after using AI compared to CON regardless of TSD. However, neither SA nor DAS were affected by AI or TSD ($p > 0.05$). Independent of AI, the SRT was slower (3.2-9.3%; $p < 0.001$) in the mornings (-24, +8) than in the evening (-12), JH was higher (3.0-4.7%, $p < 0.001$) in the evenings (-12, -36) than in the mornings (0, -24, +8), and RPE was higher (20.0-40.1%; $p < 0.001$) in the sleep-deprived morning (-24) than all other timepoints (0, -12, -36, +8). Furthermore, higher ESS (59.5-193.4%; $p < 0.001$) was reported at -24 and -36 than the rest of the timepoints (0, -12, and + 8).

Conclusion:

The literature review unveiled a notable gap in understanding the impact of ammonia inhalations on cognitive and physical performance, noting potential short-term effects on the cardiorespiratory system. A subsequent study on laser-based shooting simulations for military personnel demonstrated moderate to good reliability in static and dynamic protocols, potentially paving the way for more efficient, cost-effective and reliable testing and training methods. The final study highlighted that while total sleep deprivation negatively affects cognitive and physical performance, the application of ammonia inhalants can offer short-term improvements in reaction time, jump height, heart rate and reduced perceived exertion, irrespective of total sleep deprivation status. On the other hand, it did not affect shooting accuracy or rifle assembly and disassembly skills. These findings may present ammonia inhalants usage as a potential ergogenic aid in specific military situations. In conclusion, the combined results provide new insights into the potential impacts of ammonia inhalants and total sleep deprivation on cognitive and physical performance and underline the need for additional research to ascertain their efficacy and safety in broader military contexts.

Keywords: Ergogenic aids, Smelling salts, Firearm, Army, Sleep Loss

Abstrakt (in the Czech language)

Vzhledem k tomu, že je tato disertační práce postavena na třech na sebe navazujících studiích, jsou cíle, metody, a výsledky práce rozděleny do tří samostatných podkapitol (a, b a c), z nichž každá odpovídá jedné studii.

Název:

Vliv spánkové deprivace a uhličitanu amonného na psychické a fyzické vlastnosti členů ozbrojených složek

Cíle:

a) Vypracování přehledu literatury s cílem zaplnit podstatnou mezeru v literatuře zabývající se zkoumáním vlivu inhalace uhličitanu amonného na kognitivní a fyzickou výkonnost, a tím přispět k současným znalostem a porozumění tohoto tématu.
b) Vypracování studie s cílem prozkoumat chybějící test-retest reliabilitu standardních statických a dynamických vojenských střeleckých protokolů pro komplexní zkoumání vlivu celkové spánkové deprivace a inhalace uhličitanu amonného na přesnost střelby z ručních zbraní.
c) Vypracování kontrolní studie zaměřené na zkoumání účinnosti inhalace uhličitanu amonného na dopady 36hodinové celkové spánkové deprivace na baterii kognitivních a fyzických výkonnostních testů relevantních pro vojenský personál.

Metody:

a) Pro účely tohoto přehledu literatury byla provedena rešerše v následujících elektronických databázích: MEDLINE (PubMed), SCOPUS, Web of Science a Google Scholar. Vzhledem k nedostatečnému počtu výzkumů na toto téma byly vyhledávací termíny poměrně široké a zahrnovaly řadu pojmů včetně: amoniak, inhalace, výkon a čichací sůl. Kromě toho bylo vzhledem k nedostatku recenzovaných článků provedeno ruční vyhledávání ze seznamu literatury všech získaných článků do dubna 2020.
b) Dvacet vojáků (22 ± 1,9 let; 81,7 ± 8,6 kg; 184,9 ± 5,9 cm) se ve třech různých dnech zúčastnilo dvou testů statických a dynamických střeleckých protokolů, za účelem zjištění reliability přesnosti střelby mezi jednotlivými dny a v rámci

jednoho dne. Přesnost (celkový počet dosažených bodů) pro každý pokus byla zaznamenána a analyzována pro 1) reliabilitu každého střeleckého protokolu pomocí koeficientu vnitrotřídní korelace (ICC) s variačním koeficientem (CV) a standardní chybou měření (SEM) a 2) rozdíly v počtu dosažených bodů přesnosti střelby mezi dny a pokusy pomocí dvoucestné RM ANOVA.

c) Osmnáct kadetů (24,1 ± 3,0 let; 79,3 ± 8,3 kg) provedlo 5 identických testů během 36 hodin celkové spánkové deprivace (TSD) (po 0 [0], 12 [-12], 24 [-24] a 36 [-36] hodinách TSD) a po 8 [+8] hodinách regeneračního spánku. Během každého testování byly provedeny následující testy: Epworthská škála spavosti (ESS), jednoduchý reakční čas (SRT), přesnost střelby (SA), rozložení a opětovné složení pušky (DAS) a výška výskoku (JH). Během testu SA byla průběžně monitorována srdeční frekvence (HR) a během testu JH bylo zjišťováno hodnocení vnímané námahy (RPE). V každém časovém bodě byly testy provedeny dvakrát, a to po použití uhličitanu amonného (AI), nebo bez použití AI (CON), v randomizovaném pořadí.

Výsledky:

a) Dosud chybí důkazy, které by potvrzovaly tvrzení o zvýšeném kognitivním nabuzení a zlepšeném silovém výkonu. Může však existovat krátkodobý účinek po inhalaci uhličitanu amonném na kardiorespirační systém (zvýšení dechové frekvence a srdeční frekvence přibližně na 15 až 30 sekund), ale je zapotřebí dalšího výzkumu, který by tato zjištění potvrdil a určil, jak mohou krátkodobé kardiorespirační účinky ovlivnit další fyziologické a výkonnostní ukazatele. Závěrem, ačkoli důkazy nenaznačují, že by inhalace uhličitanu amonného byla u zdravé populace nebezpečná, měli by si sportovní a zdravotničtí odborníci být vědomi možných rizik, aby předešli případným nepravděpodobným, ale možným obtížím.

b) Výsledky ukázaly dobrou reliabilitu testů mezi jednotlivými dny u průměru dvou pokusů statického (ICC = 0,837 [0,659, 0,930], CV = 3,78 %, SEM = 3,37) i dynamického (ICC = 0,806 [0,597, 0,917], CV = 4,73 %, SEM = 3,73) protokolu. Kromě toho byla zjištěna střední reliabilita mezi jednotlivými dny testování u statického (ICC = 0,703 [0,383, 0,872], CV = 3,47 %, SEM = 3,11) a dynamického (ICC = 0,585 [0,219, 0,810], CV = 4,17 %, SEM = 3,30) protokolu a střední reliabilita stejného dne při opakovaném testování u statických

(ICC = 0,510 [0,248, 0,741], CV = 2,57 %, SEM = 2,31) a dynamických (ICC = 0,510 [0,243, 0,742], CV = 4,30 %, SEM = 3,39) protokolů v posledních dvou pokusech.

c) V žádném z testů nebyla zjištěna interakce mezi podmínkami a časem, ale po použití AI došlo k rychlejšímu SRT (1,6 %; p = 0,007) bez zvýšení počtu chyb, vyšší JH (1,5 %; p = 0,005), nižšímu RPE (9,4 %; p < 0,001) a vyššímu HR (5,0 %; p < 0,001) ve srovnání s CON bez ohledu na TSD. SA ani DAS však nebyly ovlivněny AI ani TSD (p > 0,05). Nezávisle na AI byla SRT pomalejší (3,2-9,3 %; p < 0,001) ráno (-24, +8) než večer (-12), JH byla vyšší (3,0-4,7 %, p < 0. 001) večer (-12, -36) než ráno (0, -24, +8) a RPE byla vyšší (20,0-40,1 %; p < 0,001) ráno (-24) při nedostatku spánku než ve všech ostatních časových bodech (0, -12, -36, +8). Dále byla zaznamenána vyšší ESS (59,5-193,4 %; p < 0,001) v -24 a -36 než v ostatních časových bodech (0, -12 a +8).

Závěr:

Přehled literatury odhalil výraznou mezeru v pochopení dopadu používání uhličitanu amonného na kognitivní a fyzickou výkonnost a upozornil na možné krátkodobé účinky na kardiorespirační systém. Následná studie zabývající se zjištěním chybějící reliability střeleckých protokolů pro vojenský personál pomocí laserové pistole prokázala střední až dobrou reliabilitu statických a dynamických protokolů, což potenciálně otevírá cestu k účinnějším, méně finančně náročným, a spolehlivějším metodám testování a výcviku střelby. Závěrečná studie zdůraznila, že zatímco celková spánková deprivace negativně ovlivňuje kognitivní a fyzickou výkonnost, aplikace uhličitanu amonného může nabídnout krátkodobé zlepšení reakční doby, výšky skoku, srdeční frekvence a snížení vnímané námahy bez ohledu na celkový stav spánkové deprivace. Na druhou stranu přesnost střelby ani celkový čas při rozkládání a skládání pušky nebyly uhličitanem amonným ovlivněny. Tato zjištění mohou prezentovat užívání uhličitanu amonného jako potenciální ergogenní pomůcku ve specifických vojenských situacích. Závěrem lze říci, že kombinované výsledky poskytují nové poznatky o potenciálních dopadech používání uhličitanu amonného a celkové spánkové deprivace na kognitivní a fyzickou výkonnost a zdůrazňují potřebu dalšího výzkumu, který by zjistil jejich další potencionální účinnost a bezpečnost v širším vojenském kontextu.

Klíčová slova: Ergogenní látky, Čichací sůl, Střelná zbraň, Armáda, Ztráta spánku

Content

Peer-reviewed publications included in this dissertation thesis

Chapter 4. Malecek J, Tufano JJ. *Effects of Ammonia Inhalants in Humans: A Review of the Current Literature Regarding the Benefits, Risks, and Efficacy*. Strength and Conditioning Journal, 2021. doi: 10.1519/SSC.0000000000000630

Chapter 6. Malecek J, Omcirk D, Didek Z, Michalicka V, Sykora K, Vagner M, Privetivy L, Trebicky V, Vetrovsky T, Tufano JJ. *Test-Retest Reliability of Two Different Laser-based Protocols to Assess Handgun Shooting Accuracy in Military Personnel*. BMJ Military Health, 2023. doi: 10.1136/military-2023-002431

Chapter 8. Malecek J, Omcirk D, Skalova K, Padecky J, Janikov MT, Obrtel M, Jonas M, Kolar D, Michalicka V, Sykora K, Vagner M, Privetivy L, Vetrovsky T, Bendova Z, Trebicky V, Tufano JJ. *Effects of 36 hours of sleep deprivation on military-related tasks: can ammonium inhalants maintain performance?* PLOS ONE, 2023 (Under review, (Appendix 1).

Peer-reviewed abstracts from conferences linked to this dissertation thesis

Malecek J, Omcirk D, Padecky J, Bendova Z, Skalova K, Kolar D, Privetivy L, Sykora K, Vagner M, Tufano JJ. *Effect of total sleep deprivation and ammonia inhalants on reaction time in military personnel.* 6th International Congress on Soldiers' Physical Performance, London, UK, September 2023 (Appendix 2).

Malecek J, Omcirk D, Padecky J, Bendova Z, Skalova K, Kolar D, Privetivy L, Sykora K, Vagner M, Tufano JJ. *Effect of total sleep deprivation and ammonia inhalants on rating of perceived exertion in military personnel.* National Strength and Conditioning Association Annual Conference, Las Vegas, NV, USA, July 2023 (Appendix 3).

Malecek J, Omcirk D, Padecky J, Bendova Z, Skalova K, Kolar D, Privetivy L, Sykora K, Vagner M, Tufano JJ. *Effect of total sleep deprivation and ammonia inhalants on countermovement jump performance in military personnel.* National Strength and Conditioning Association Annual Conference, New Orleans, LA, USA, July 2022. Journal of Strength and Conditioning Research 37(3):p e25-e272, March 2023. doi: 10.1519/JSC.0000000000004416 (Appendix 4).

Malecek J, Omcirk D, Padecky J, Bendova Z, Skalova K, Kolar D, Privetivy L, Sykora K, Vagner M, Tufano JJ. *Effect of total sleep deprivation and ammonia inhalants on handgun shooting accuracy in military personnel.* National Strength and Conditioning Association Annual Conference, New Orleans, USA, July 2022. Journal of Strength and Conditioning Research 37(3):p e25-e272, March 2023. doi: 10.1519/JSC.0000000000004416 (Appendix 5).

Malecek J, Omcirk D, Didek Z, Vetrovsky T, Tufano JJ. *Reliability of a marksmanship test while using a laser-based marksmanship simulator system in military personnel.* National Strength and Conditioning Association Annual Conference, Orlando, FL, USA, July 2021 (Online). Journal of Strength and Conditioning Research: December 2021 - Volume 35 - Issue 12 - p 291-446. doi: 10.1519/JSC.0000000000004141 (Appendix 6).

1. Introduction

Recent warfare events necessitated an institutional evolution in the way military leaders look at the soldiers' health as an essential part of performance and readiness. Throughout the past years, the Ministry of Defense & Armed Forces of the Czech Republic has demonstrated a heightened interest in service members' physical and mental preparedness (Bugala, 2020). This is mainly apparent in the joint efforts between command and healthcare workers, who work together to improve the well-being habits of service members in all environments, from training to operations and combat. Placing emphasis on nutrition, correctly chosen physical activity, mental resilience training, fatigue management, sleep hygiene etc., seeks to mitigate the potentially harmful side effects of maintaining a persistently ready force across the entire defense establishment. However, sleep is often an overlooked component (Good et al., 2020).

Sleep is a crucial yet frequently undervalued biological process that is widely recognized as critical for military personnel's health and combat readiness (Thompson et al., 2017). However, self-reported data suggests that military personnel obtain less sleep than the recommended 7.5 to 8.5 hours per day (Meadows et al., 2018; Van Dongen et al., 2003; Wehr et al., 1993), potentially affecting their ability to perform military duties effectively (Heaton et al., 2014). In addition, military personnel often face situations where they are required to perform continuous tasks for up to 24 hours, such as overnight duty, prolonged operations, or direct ground combat (Reynolds & Banks, 2010). Under such operational circumstances, soldiers may experience total sleep deprivation, a lack of sleep that surpasses 24 hours (Reynolds & Banks, 2010).

Based on previous research, total sleep deprivation has been found to decrease blood flow velocity in the middle cerebral artery, which can lead to various cognitive and behavioral impairments, including fatigue, sustained attention, and reaction time (Csipo et al., 2021). Manual dexterity, a critical component of tasks such as shooting and firearms handling, has also been shown to be negatively impacted by total sleep deprivation (Dąbrowski et al., 2012; Hirkani & Yogi, 2017; Tharion et al., 2003). Furthermore, total sleep deprivation not only impairs cognitive functioning and perceptual-motor skills but also diminishes short-term, high-intensity exercise output (Dąbrowski et al., 2012; Skein et al., 2010; Souissi et al., 2013; Tomczak, 2015).

As we consider the broad problematic of total sleep deprivation, it is required to shift our focus back to specific groups where the effect of insufficient rest may be more acute. Military personnel are a unique, urgent, and understudied group to investigate, as total sleep deprivation and insufficient rest are operationally critical (Good et al., 2020; United States, Department of Defense, 2021).

Given that soldiers frequently engage in high-intensity, short-term movements and rely heavily on their ability to react swiftly, handle weapons, and shoot accurately, any decline in these performance areas, a potential consequence of sleep deprivation, may negatively impact their survival and combat effectiveness (Friedl et al., 2015; Good et al., 2020). As the potential risks and high-stakes nature of military service make impaired sleep a significant concern, understanding its detrimental effects on both cognitive and physical functioning is crucial (Harman et al., 2008).

As such, ergogenic aids, which may mitigate the adverse effects of sleep deprivation by promoting cognitive and physical performance, are of high interest to the military (Harman et al., 2008). For instance, caffeine, one of the most widely used ergogenic aids, has been found to help sustain cognitive functioning and enhance physical performance during total sleep deprivation at moderate doses (Crawford et al., 2017; McLellan et al., 2016). However, caffeine cannot replace regular sleep, and excessive consumption can disrupt regular sleep habits (Chaput et al., 2020; Good et al., 2020; Wesensten et al., 2011). Furthermore, while the effects of caffeine may last for several hours, its onset is typically observed within a few tens of minutes post-consumption, reflecting the required digestion time (Graham, 2001). Consequently, its utility for immediate performance enhancement might be limited. Hence, ergogenic aids characterized by a more rapid onset of action may prove more advantageous in the circumstances demanding immediate soldier arousal for physical or cognitive performance.

An example of fast-acting ergogenic aids is ammonia inhalants, which are traditionally used as a fast-acting "pre-workout stimulant", whereby users hope for rapid improvements in vigilance and short-term high-intensity physical performance (McCrory, 2006). The putative effect of arousal via ammonia inhalants inhalation is believed to be caused by irritation of the respiratory passages that may subsequently trigger the adrenergic receptors in peripheral tissue, resulting in the release of norepinephrine, causing an increase in cardiac output, respiratory rate and an increase in blood flow velocity in the middle cerebral artery (Perry et al., 2016).

Although there is no evidence suggesting that ammonia inhalants usage affects maximal muscular strength or endurance, some studies indicate that it enhances alertness, laboratory-tested explosive strength during isometric muscle actions, and repeated anaerobic power performance when athletes are already fatigued (Campbell et al., 2022; R. R. Rogers et al., 2022; Secrest et al., 2015). Moreover, a short-term increases in heart rate, breath rate (Campbell et al., 2022; Perry et al., 2016), and blood flow velocity of the middle cerebral artery (Perry et al., 2016) has been observed after inhaling ammonia inhalants. However, no effects have been observed in real-world movements and tasks such as jump height or sprint time (Malecek & Tufano, 2021). To conclude, despite their widespread use among some athletes, the literature lacks evidence on the effects of ammonia inhalants usage on cognitive and physical performance. Furthermore, no prior research appears to have investigated the effects of these inhalants on sleep-deprived individuals.

Therefore, this dissertation thesis focuses on examining the effectiveness of using ammonia inhalants in countering the effects of total sleep deprivation on cognitive and physical performance tests relevant to military personnel, whereas the main objectives of this dissertation are:

- Conducting a literature review to identify the current understanding on the topic and substantial gaps in the literature by investigating the impact of ammonia inhalations on cognitive and physical performance.
- Conducting a methodological study to examine lacking test-retest reliability of standard-issued static and dynamic military shooting protocols for comprehensive investigation of the of total sleep deprivation impact and ammonia inhalants on handgun shooting accuracy.
- Conducting a control trial study to investigate the effectiveness of ammonia inhalants in altering the impact of 36 hours of total sleep deprivation on battery of cognitive and physical performance tests relevant to military personnel.

This dissertation thesis comprises 9 chapters, with Chapter 1 serving as the fundamental introduction, providing a broad overview and aims of the dissertation thesis and establishing a foundation for the subsequent chapters. Chapter 2 reviews the current knowledge on sleep, sleep deprivation, and related ergogenic aids, specifically focused on the military environment. This chapter aims to establish the theoretical foundation for the three studies that form the core of this dissertation thesis and which were published (or submitted) as three individual studies in peer-reviewed journals.

To facilitate a cohesive presentation of the studies, the methods, results, and discussions for each study are presented in Chapters 4, 6 and 8. In addition, each of the three studies is briefly introduced (Chapter 3, 5 and 7) to link the chapters together and show how each study's findings contribute to one another. Lastly, Chapter 9 provides an overall summary of the entire dissertation thesis and my doctoral studies.

Chapter 4 comprises the manuscript titled "*Effects of Ammonia Inhalants in Humans: A Review of the Current Literature Regarding the Benefits, Risks, and Efficacy",* which was published in 2021 in the Strength and Conditioning Journal (IF = 2.490, Q3 in Sport Sciences). The manuscript thoroughly reviews the available literature on using ammonia inhalants in humans. The study specifically explores the potential benefits, risks, and efficacy associated with ammonia inhalant usage and provides a comprehensive overview of current knowledge on this topic (Malecek & Tufano, 2021).

Chapter 6 includes the manuscript titled "*Test-Retest Reliability of Two Different Laser-based Protocols to Assess Handgun Shooting Accuracy in Military Personnel*", which was published in 2023 as a Letter to Editor in the BMJ Military Health journal (IF = 2.800, Q2 in Medicine, General & Internal). The manuscript highlights the importance of establishing the reliability of basic shooting protocols employed in research to assess the impact of various factors on shooting accuracy and presents original results of the reliability of selected static and dynamic shooting protocols performed using a laser-based handgun system (Malecek et al., 2023).

In Chapter 8, the manuscript titled "*Effects of 36 hours of sleep deprivation on military-related tasks: can ammonium inhalants maintain performance?*" submitted in 2023 and currently under review in the PLOS ONE journal (IF = 3.752, Q2 in Multidisciplinary), reports the potential impact of the use of ammonia inhalants on various measures, including simple reaction time, shooting accuracy, countermovement jump height, and rifle disassembly, and reassembly time, in military personnel subjected to total sleep deprivation of 36 hours, followed by a recovery sleep period of 8 hours. The outcomes of the manuscript indicate that implementing ammonia inhalants could potentially have some practical implications in specific military scenarios, regardless of the duration of the total sleep deprivation.

In conclusion, it is necessary to mention that this dissertation thesis was made possible through the generous support of the Charles University Grant Agency. The project received funding under grant number GAUK 986120. This invaluable backing not only enabled us to conduct rigorous research but also significantly contributed to our ability to explore and contribute new knowledge in this essential field. We are deeply grateful for this support and recognize its critical role in the success of our endeavor.

2. Theoretical background

This chapter aims to introduce and review the general physiology of sleep and sleep deprivation with its potential subsequent physiological consequences. We are laying the groundwork by elucidating the concept of sleep, delving into its intricate architecture, and distinguishing between both the non-rapid eye movement and rapid eye movement sleep phases. These two states of sleep exhibit distinctive characteristics and functions integral to our understanding of sleep.

Moreover, we are introducing the systems that control sleep and circadian rhythms. These regulatory mechanisms are essential in maintaining the delicate balance between sleep and wakefulness, affecting numerous aspects of our physiology and behaviour.

The second part of this chapter concentrating on the ramifications of sleep deprivation, both partial and total, particularly as it relates to the military environment. We assess its impact on military personnel's cognitive and physical abilities, which we are trying to provide a comprehensive picture of its broad-ranging effects.

Lastly, we examine ergogenic aids as a potential mitigating factor against the potential adverse effects of sleep deprivation. These substances, designed to enhance cognitive and physical performance, may possibly offer some help against the negative effects of sleep deprivation, allowing soldiers to maintain performance despite sleep deficits.

This chapter should provide a broad overview, aiming to give the reader a robust understanding of the role of sleep with a focus on the military context.

2.1. Sleep definition

Humans dedicate approximately one-third of their existence to sleep, but most possess limited knowledge about this essential physiological process (Aminoff et al., 2011). Despite the precise functions of sleep remaining incompletely understood, it constitutes a fundamental requirement for higher organisms, including humans, with its deprivation leading to significant physiological consequences (Colten et al., 2006).

Sleep is a fundamental physiological process crucial for preserving physical and mental well-being (Medic et al., 2017). It is a natural, recurring state involving the mind and body, marked by altered consciousness, diminished sensory activity, and reduced muscle activity (Zisapel, 2007). Sleep is vital in brain operations and systemic physiology across numerous bodily systems (Medic et al., 2017). The body engages in intricate physiological processes throughout sleep, such as energy restoration, memory consolidation, and immune function regulation (Imeri & Opp, 2009). Sleep is also essential for managing various biological processes, including metabolism, hormone secretion, and gene expression (Van Cauter et al., 2008). The biological significance of sleep becomes apparent when considering that lack of sleep can result in various adverse health consequences, including cognitive deficits, mood disorders, and metabolic dysfunction (Knutson, 2007). As a result, it is imperative to encourage proper sleep hygiene practices in both genders, across various age groups, and among individuals from diverse professional backgrounds, as adequate sleep is crucial for maintaining optimal performance and overall well-being in all aspects of life (Knutson, 2007; Medic et al., 2017; Van Cauter et al., 2008; Zisapel, 2007).

Despite the common understanding of sleep as an altered state of consciousness, its definition and functions have consistently puzzled researchers. The progression of the deafferentation hypothesis of sleep has traditionally been tied to a concept more than before 2000 years back, where it was proposed that sleep is essentially the state of non-wakefulness (Moruzzi, 1964). In 1749 (Hartley, 1749) and 1834 (Macnish, 1834), his notion underwent further developments, defining sleep as a period where sensory abilities are paused and voluntary actions cease. However, involuntary processes like respiration and circulation carry on without interference (Chokroverty, 2010). Understanding the nature of sleep becomes more apparent when one poses the question while attempting to fall asleep. Human sleep may be described as a modified state characterized by the diminished conscious awareness of the external environment, accompanied by distinct controls, rhythms, emotions, and dreams (Chokroverty, 2010). This transient, natural, and periodic physiological occurrence is reversible, thereby setting it apart from irreversible states such as coma and death (Chokroverty, 2017a).

Two components are necessary for consciousness: arousal, facilitated by the ascending reticular activating system, and awareness, which is a function of the cerebral cortex (Machado, 1999). Pathological conditions such as coma or unconscious state are different from sleep in several ways. Sleep, distinct from a coma, results in only slight changes in brain metabolism and circulation, whereas a coma presents significant depression and impairment in these areas (Chokroverty, 2017b). Despite some surface resemblances, states such as coma, persistent vegetative state, and minimally conscious state are fundamentally different from sleep (Chokroverty, 2017b). Recent researchers delineate sleep based on the individual's asleep behaviour and the corresponding physiological changes in the waking brain's electrical rhythm during sleep (Ogilvie, 2001). Behavioral markers of sleep include calmness, limited movement, specific sleep postures, closed eyes, reduced responsiveness to stimuli, impaired cognitive abilities, slower time of reaction and returnable unconsciousness (Chokroverty, 2010, 2017b).

Also, simply put, sleep may be defined as a reversible behavior state marked by detachment from and unresponsiveness to the environment (Carskadon & Dement, 2011). Sleep is also a multifaceted combination of physiological and behavioral states (Carol, 2011). Generally, sleep is associated with (though not exclusively) a recumbent posture, subdued behavior, closed eyes, and other features commonly linked to slumber (Carskadon & Dement, 2011). In atypical circumstances, additional behaviors may transpire during sleep, encompassing sleepwalking, sleep talking, teeth grinding, and various physical activities (Carskadon & Dement, 2011). Sleep process anomalies also comprise instances of sleep intrusions, be it the act of sleeping itself, dream imagery, or muscle weakness within waking hours (Carskadon & Dement, 2011).

Sleep serves as an active period of anabolism, aiding in growth and enhancing the immune system, and is a phenomenon also observed in birds, mammals, amphibians, fish, and reptiles (Chokroverty, 2017b). Sleep is defined physiologically by data from electroencephalography, electrooculography, electromyography, and changes in breathing and blood flow (Chokroverty, 2017b). When trying to understand the onset of sleep, it is vital to differentiate between feelings of sleepiness and sensations of fatigue or weariness (Chokroverty, 2017b). Unlike sleepiness, which manifests as heavy eyelids, yawning, head nodding, or the ability to nap if the opportunity arises, fatigue is defined by a constant state of low energy and diminished motivation (Chokroverty, 2009). On the other hand, sleepiness often results in fatigue as a secondary outcome (Chokroverty, 2009, 2017a).

2.2. Sleep architecture and sleep profile

Sleep may be differentiated into non-rapid eye movement, and rapid eye movement sleep which are both characterized by distinct functionalities and controls, as identified through three physiological evaluations (previously mentioned): electroencephalography, electrooculography, and electromyography (Chokroverty, 2009, 2010). Ideally, though not consistently observed in all normal individuals, non-rapid eye movement and rapid eye movement sleep alternate cyclically, averaging between 90 and 110 minutes per cycle (Colten et al., 2006). In a typical adult sleep cycle, 4-6 stages occur. The initial two cycles are characterized by predominant slow-wave sleep, whereas the following stages contain less slow-wave sleep, which may even disappear entirely in certain instances (Chokroverty, 2017b).

On the other hand, the duration of the rapid eye movement sleep cycle extends by every additional cycle, climaxing in a potentially hour-long rapid eye movement sleep episode at the end (Chokroverty, 2017b). As a result, adult human sleep is characterized by slow-wave sleep dominating the initial third and rapid eye movement sleep dominating the final third (Dijk, 2009). It is vital to acknowledge these features, as specific abnormal motor activities are typically linked to slow-wave sleep and rapid eye movement sleep (Chokroverty, 2017a). This sleep architecture embodies the fundamental structural composition of regular sleep, with non-rapid eye movement sleep further diverged into four stages, demonstrating a spectrum of sleep's depth (Chokroverty, 2017b). Distinct characteristics, such as patterns of brain waves, variations in muscle tone, and eye movements, are exhibited in each stage (Chokroverty, 2009). The identification of sleep cycles and stages was facilitated by the use of electroencephalography recordings, which capture the brain's electrical activity patterns (Dement & Kleitman, 1957; Loomis et al., 1937).

2.2.1. Non-rapid eye movement sleep

Given that non-rapid eye movement sleep comprises about 75 to 80% of an adult human's total sleep duration, it is important to note that according to the Rechtschaffen and Kales scoring manual, this type of sleep is further divided into four distinct stages, labelled 1 to 4 (Institute of Medicine, 2006). Primarily based on electroencephalography patterns, the scoring manual by the American Academy of Sleep Medicine which is more recently published and used, delineates three stages (N1, N2, and N3), offering a comparison to the four stages outlined in the older Rechtschaffen and Kales scoring manual (Moser et al., 2009; Novelli et al., 2010). N1 sleep, or Stage 1, represents 3 to 8% of total sleep time, while N2, or Stage 2, accounts for 45 to 55% of the sleeping period, and N3, also known as slow-wave sleep, constitutes 15–23% of the overall sleep duration (Chokroverty, 2017b). In awake adults, the dominant rhythm is the alpha rhythm (8–13 Hz), mainly seen in the posterior head regions, with a slight presence of the beta rhythm (>13 Hz) in the anterior regions (Chokroverty, 2009). During the wakeful state, known as stage W, eye movements may be observed, including vertical, horizontal, slow, oblique, or rapid eye movements (Chokroverty, 2017b).

a) Stage 1

Stage 1 (N1) sleep, also known as light sleep (Patel et al., 2023), functions as an intermediary step within the sleep-stage progression, signifying the onset of sleep for most individuals, with the exception of those affected by narcolepsy, particular neurological conditions or infants (Carskadon & Dement, 2011). This initial stage, highly susceptible to interruptions from external disturbances, constitutes 2 to 5% of the total sleep duration and typically lasts for a brief span of 1 to 7 minutes (Carskadon & Dement, 2011). Electroencephalography brain activity shifts from the wakeful state, which is denoted by rhythmic alpha waves indicative of a relaxed yet alert condition (8 to 13 cycles per second), to a pattern characterized by low-voltage, mixed-frequency waves, as non-rapid eye movement sleep commences in stage 1 (Carskadon & Dement, 2011). During this phase, the alpha rhythm drops below 50% in an epoch, also slower theta and delta electroencephalography rhythms (1 to 7 Hz) emerge (Louis et al., 2016), electromyographic activity slightly decreases, slow eye movements begin to manifest, and vertex sharp waves become noticeable, all occurring simultaneously as the stage draws near its end (Chokroverty, 2017b).

a) Stage 2

Commencing with a duration of around 10 to 25 minutes, Stage 2 (N2) sleep gradually lengthens across subsequent cycles, accounting for 45 to 55% of the overall sleep time eventually, indicating a deeper sleep state which requires a more potent stimulus to awake an individual in comparison to stage 1 sleep (Institute of Medicine, 2006). As detected by electroencephalography, Stage 2 sleep is described by mixed-frequency brain activity (moderately low-voltage), including the K-complexes, sleep spindles (Ioannides et al., 2019), and interspersed vertex sharp waves (Gais et al., 2002; Institute of Medicine, 2006). Previous research showed that sleep spindles are postulated to be crucial in memory functioning, as demonstrated by a higher spindle density in individuals who engage in new tasks (Gais et al., 2002). Moreover, during stage 2 sleep, the electroencephalography recordings also reveal the presence of theta and slow waves (from 0.5 to 2 Hz), that account for under 20% of the epoch's total duration (Chokroverty, 2009).

b) Stages 3 and 4 (slow-wave sleep)

Primarily occurring during the first third of the night, Stages 3 (N3) and 4 (slow-wave sleep) encompass slow-wave sleep, which is distinguished by its specific attributes (Chokroverty, 2017b). Subsequent to 30 to 60 minutes of stage 2 (N2) sleep, stage 3 initiates, with slow waves accounting for 20 to 100% of the epoch. Rechtschaffen and Kales scoring manual stages 3 and 4 of non-rapid eye movement sleep, collectively known as slow-wave sleep, are superseded by stage N3 in the revised American Academy of Sleep Medicine scoring manual (Institute of Medicine, 2006). Displaying enhanced high-voltage, slow-wave activity in electroencephalogram readings, Stage 3 sleep, which lasts only a couple minutes and represents about 3 to 8% of total sleep, is another crucial part of the sleep cycle (Institute of Medicine, 2006). Following Stage 3, the concluding phase of non-rapid eye movement sleep, Stage 4, which spans approximately 20 to 40 minutes in the first cycle and constitutes around 10 to 15% of total sleep, showcases increased high-voltage, slow-wave activity on the electroencephalogram and holds the distinction of having the highest arousal threshold (require more intense stimuli to awake individuals) among all non-rapid eye movement sleep stages (Carskadon & Dement, 2011). Body movements, frequently recorded as artefacts in polysomnographic data, intensify as slow-wave sleep lightens and approaches its ending. The transition to the initial rapid eye movement sleep period, which occurs roughly 60 to 90 minutes after sleep onset, is preceded by a brief interruption of stage 3 by stage 2 (Chokroverty, 2017a).

2.2.2. Rapid eye movement sleep

Characterized by desynchronized brain wave activity identical to a wakeful state, muscle atonia, and visible rapid eye movements, rapid eye movement sleep presents a unique and essential sleep stage (Kryger et al., 2005), accounting for 20 to 25% of total sleep time (Yamada & Ueda, 2020). The loss of muscle tone and reflexes plays a crucial role in rapid eye movement sleep by preventing individuals from physically reacting to their dreams or nightmares (which may prevent from possible injuries) (Bader et al., 2003; Chokroverty, 2017b). This sleep stage, which may also play a significant role in memory functioning (Crick & Mitchison, 1983; C. Smith & Lapp, 1991), is where approximately 80% of dream recall happens upon awakening (Dement & Kleitman, 1957). During rapid eye movement sleep, phases of apnea (pause in breathing) or hypopnea (period of shallow breathing) might take place, often punctuated by sporadic couple seconds-long appearances in electroencephalographic' alpha rhythms

(Chokroverty, 2009). During rapid eye movement sleep, the brainwave activity shown on electroencephalographic recordings presents a fast and low amplitude pattern within the beta frequency range, with several of theta rhythms that sometimes display a "sawtooth" pattern (Chokroverty, 2017b). These waves, typically fluctuating between 2 to 6 Hz and often irregular, are predominantly detected in the central areas of the brain and are supposed to signify the onset of rapid eye movement sleep, usually preceding its initiation (Chokroverty, 2017b). The initial cycle of rapid eye movement sleep, lasting around 5 minutes, then progressively extending throughout the overall sleep, transitions to Stage 2 (N2) and subsequently to Stage 3 (N3) before giving way to the second occurrence of the rapid eye movement sleep phase (Chokroverty, 2009; Kryger et al., 2005).

2.3. Sleep rhythms

Sleep rhythms, also referred to as circadian rhythms, are innate mechanisms that control the sleep-wake cycle and various other bodily functions in an approximately 24-hour time span (Duffy et al., 2011). The anterior hypothalamus houses the suprachiasmatic nucleus, which is the principal regulator of these rhythms (Ralph et al., 1990). The suprachiasmatic nucleus influences numerous behavioral and physiological cycles through its neural and hormonal signals, prominently affecting temperature control (Waterhouse et al., 2005), gene expressions (Dunlap, 1999), and hormonal secretions (Gehrman et al., 2013; Sack et al., 2000). Additionally, external stimuli like light and social cues play a role in aligning these circadian rhythms (Dijk et al., 2012; Duffy et al., 2011; Edwards et al., 2009; Facer-Childs et al., 2018).

Typically known as their chronotype, a person's natural preference for morning or evening activities can be classified into "larks" or early chronotypes, and "night owls" or late chronotypes (Roenneberg et al., 2003). Larks have significantly earlier sleep-wake cycles in comparison to night owls, who are more inclined to be functional later in the day. This divergence is not limited to sleep behaviors but also affects various 24-hour oscillations, including physiological, behavioral, and genetic ones (Bailey & Heitkemper, 2001; Roenneberg et al., 2003; Takahashi et al., 2008).

Previous research suggests that the cyclical nature of physiological and behavioral processes of circadian rhythms may influence with times of individuals' peak performances (Facer-Childs et al., 2018). The prevalent consensus among researchers is that late afternoon to early evening, typically between 16:00 to 18:00 (Kline et al., 2007), when the core body temperature is at its maximum, represents the most favorable time for athletic performance; this is underpinned by the theory that a higher core body temperature helps actin-myosin cross-bridging in skeletal muscles, thereby may leading to improve performance (Teo et al., 2011). Conversely, when the core body temperature is at its lowest, typically around 03:00 (Waterhouse et al., 2005), it is implied that performance can be compromised.

Even though previous research suggests the early evening is the peak time for the physical aspects of athletic performance, studies have reported superior accuracy (Atkinson & Speirs, 1998), fine motor control, and short-term memory performance in the morning (Drust et al., 2005). However, research in sports like soccer or tennis has shown that other technical skills perform better in the afternoon or evening (Thun et al., 2015), which adds to the ongoing controversy surrounding the ideal timing for maximal cognitive performance during the 24-hour rhythm.

In conclusion, circadian rhythms may have a profound influence on cognitive and physical performance (Fisk et al., 2018). Additionally, potential disruptions in circadian rhythms (e.g., traveling or night shifts) can lead to cognitive and physical deficits, poorer mental health, and increased health risks (Sletten et al., 2020; Walker et al., 2020). Therefore, understanding the relationships between the effects of proper sleep hygiene and circadian rhythms on cognitive and physical performance is crucial for optimizing health, well-being, and performance (Irish et al., 2015; Vitale et al., 2019).

2.4. Sleep deprivation

Sleep deprivation is commonly characterized in the academic literature as *"receiving insufficient sleep to ensure adequate alertness during the day"* (Kryger et al., 2017). It is well known that around a third of life is devoted to sleeping for the majority of people, yet its actual purpose remains a subject of ongoing debate in the world of science (Killgore, 2010). However, by setting aside the scientific perspective shortly, it is widely acknowledged across all populations that impaired sleep can cause our conscious actions to become significantly more difficult, demanding, and emotionally unsatisfying (Killgore, 2010). The primary significance of sleep becomes apparent when we spend the whole nighttime without it. Lack of adequate sleep typically leaves us exhausted and tired, with a noticeable decrease in our mood, often skewing towards the negative, and a perceptible slow-down in our cognitive processes (Killgore, 2010). Even though it is widely understood that sleep is critical for maintaining optimal physical and cognitive performance, sleep can sometimes be perceived as a minor inconvenience, such as a boring part of the daily routine. In other words, sleep might often seem like a period of unproductive time that could be redirected towards more effective, profitable, or enjoyable activities (Killgore, 2010). In circumstances where responsibilities related to a job, education, or social commitments become pressing, individuals frequently sacrifice sleep to allocate more time to these duties/activities (Killgore, 2010).

Moreover, lack of sleep is common in many professions, including military personnel, medical practice, and all people working in shifts, with reports suggesting that a substantial 20% of the adult population is experiencing inadequate sleep (Abrams, 2015). Therefore, the research into the consequences of sleep deprivation holds promise for distinct revelations about sleep's nature and function and also bears practical importance, particularly in enhancing the health and well-being of those who must sustain high-level performance in spite of limited or non-existent sleep (Killgore, 2010).

Expanding upon the basic concept, sleep deprivation, a condition that arises from insufficient sleep and can significantly impact cognitive and physical functioning, can be classified into two distinct categories, each with its unique characteristics and effects on the human body (Yousefpour et al., 2019).

Partial sleep deprivation, also known as sleep restriction, is a condition that describes a situation where an individual obtains some sleep, albeit less than what is generally considered sufficient, within a span of a 24-hour period. This type of sleep deprivation often occurs due to various lifestyle choices, service duty or circumstances that result in a shortened sleep duration, leading to sub-optimal rest and potential adverse health effects (Gosselin et al., 2017; United States, Department of Defense, 2021).

Total sleep deprivation, in a more comprehensive definition, is a state that is characterized by an absolute absence of sleep across a full 24-hour cycle. This condition implies that an individual does not experience any form of sleep or brief rest (e.g., short naps) throughout the day and night. It often results from extraordinary circumstances (e.g., night shifts or service duties) or specific research settings and is known to cause significant physiological and cognitive changes (Gosselin et al., 2017; United States, Department of Defense, 2021).

2.4.1. Partial sleep deprivation

Partial sleep deprivation is commonly described as experiencing no less than single night of incomplete or disturbed sleep (Kryger et al., 2017). Even though the quantity of sleep required can differ from person to person, for adults, partial sleep deprivation is generally characterized as receiving less than 5 (Pilcher & Huffcutt, 1996) to 7 hours (Gosselin et al., 2017) of sleep within a 24-hour period. In addition to partial sleep deprivation, individuals can also endure chronic partial sleep deprivation. This form of sleep deprivation can be characterized by a period lasting at least one week, during which a person consistently obtains less than 7 hours of sleep per night. Despite efforts, they are unable to secure adequate recovery sleep time to compensate for the accumulated sleep deficit (United States, Department of Defense, 2021). Often also referred to as sleep restriction, partial sleep deprivation may be more prevalent than total lack of sleep, and its impact, which can be felt with just an hour less sleep per night, and additionally becomes significantly even more severe when sleep is limited to under 4 to 5 hours each night (Williamson et al., 2011).

Consistent exposure to partial sleep deprivation can lead to various cognitive and behavioral impairments, such as loss of focus, mood declines, restricted working memory, and delayed reaction times. These effects can compound over a few days to levels equivalent to experiencing one to three consecutive nights without sleep (Banks & Dinges, 2007; Fullagar et al., 2015; Gosselin et al., 2017). It was shown that long-term memory, executive functioning, and sustained attention are primarily impacted by partial sleep deprivation (Lowe et al., 2017). However, at the same time, proofs of its effects on multitasking, intelligence, decision-making, and problem-solving are insufficient for now (Lowe et al., 2017).

Additionally, while sleep loss equals or exceeds 5 hours per night, the total impact of partial sleep deprivation on cognitive functions is more significant, leading to an 11% reduction (Lowe et al., 2017). This is in comparison to a 2 to 5 hours sleep deficit, which leads to a 9% reduction, and a sleep reduction of less than 2 hours, leading to a 3% reduction (Lowe et al., 2017). This demonstrates that the total time of partial sleep deprivation may directly affect how quickly neurocognitive deficiencies develop over a period of time when sleep is restricted, whereby both sustained attention and general cognitive ability were affected (Lowe et al., 2017).

Furthermore, the evidence demonstrated that over the span of a week, when individuals obtained 5 to 6 hours of sleep each night, they underwent a daily decrement of 1% in task accuracy. Conversely, when their nightly sleep was shortened to only 3 to 4 hours, this decline in task accuracy raised to 7% for each successive night of partial sleep deprivation (Wickens et al., 2015).

Additionally, the overall decline associated with partial sleep deprivation might be influenced by certain demographic characteristics (Lowe et al., 2017). As age advances, cognitive impairments due to partial sleep deprivation noticeably escalate within the demographic relevant to the military (Lowe et al., 2017). However, compared to men, partial sleep deprivation appears to have a reduced impact on the ability of women to maintain awareness (Lowe et al., 2017).

In this context, it becomes imperative to understand that the effects of partial sleep deprivation can be multifaceted, varying by demographic and likely influenced by a myriad of factors such as circadian rhythms (McEwen & Karatsoreos, 2015). Our understanding of these dynamics could be significantly enhanced through future investigations. A potential track for forthcoming research could be to explore the reasons behind the significant impact of partial sleep deprivation on mood and cognitive performance. For instance, the alteration of specific circadian rhythm effects on mood and performance could be caused by partial sleep deprivation. Even though the interaction between total sleep deprivation and circadian rhythms (Monk et al., 1985; Naitoh et al., 1985) has been explored in some studies, the impact of partial sleep deprivation on these rhythms remains understudied (Chokroverty, 2009). Furthermore, partial similarities between partial sleep deprivation and fragmented sleep were found, as in both scenarios, subjects manage to get at least some amount of sleep (United States, Department of Defense, 2021). Given that sleep fragmentation has been proven to impair mood and performance substantially (Bonnet, 1989), it could be suggested that the consequences of partial sleep deprivation may align more closely with the outcomes observed in sleep fragmentation rather than the effects noticed in total sleep deprivation (Durmer & Dinges, 2005).

In conclusion, considering the relative prevalence of partial sleep loss in our society, it becomes crucial to delve deeper into understanding the impacts of partial sleep deprivation. The need for a more comprehensive investigation is quite evident, given its frequency among the general population. Despite these considerations, our primary focus is total sleep deprivation, a condition we frequently encounter in our service with the Czech military training.

2.4.2. Total sleep deprivation

Characteristic of total sleep deprivation is a continuous period of being awake for more than 24 hours or not having any continuous sleep during the usual sleep-wake cycle period (Reynolds & Banks, 2010; Wickens et al., 2015). The impact of total sleep deprivation on human functions was first documented more than 100 years ago (Patrick & Gilbert, 1896). These early studies involved extended periods of sleep deprivation, such as 90 hours, and identified notable adverse effects on reaction time or memory (Patrick & Gilbert, 1896).

Given that numerous people, such as nurses, truck drivers, medics, airline pilots, or military personnel, routinely encounter total sleep deprivation due to shift work, it remains the most frequently used method for sleep disruption in laboratory research (Reynolds & Banks, 2010). Even though total sleep deprivation might not be typical among the general population (except for specific groups mentioned above), it frequently occurs in military environments due to service-duty requirements such as night shifts, prolonged activities, or direct combat, particularly during times of war (H. R. Lieberman et al., 2006).

From the safety-sensitive nature of these individuals, it is crucial to accurately evaluate how total sleep deprivation may affect cognitive functions and physical abilities and identify possible ways to mitigate these effects (Reynolds & Banks, 2010). As such, the subsequent sections briefly explore the consequences of total sleep deprivation on military personnel's cognitive and physical capabilities.

a) Effects of total sleep deprivation on cognitive functions

Previous research involving human subjects indicates that nearly every cognitive aspect is adversely influenced by the total absence of sleep (Durmer & Dinges, 2005). Furthermore, even though cognitive function impairments (e.g., prolonged reaction times), which can escalate safety threats, have been showcased across diverse professions following text will predominantly emphasize the impacts on military personnel (James & Vila, 2015; Kendall et al., 2006; H. R. Lieberman et al., 2005).

It was shown that there is a significant relationship, as demonstrated by research on military personnel, between total sleep deprivation and a considerable decline in cognitive performance related to military tasks (United States, Department of Defense, 2021). Whereby approximately 25-35% degradation in cognitive task performance can be expected with every 24 hours of completed total sleep deprivation (Buguet et al., 2003; Caldwell & Caldwell, 2005). Furthermore, the detrimental effects of total sleep deprivation intensify as individuals undergo prolonged periods of wakefulness, leading to worsening in various cognitive areas such as speed of processing, concentration, accuracy, memory, option-choosing, and reaction time (Lim & Dinges, 2010). With each passing day of total sleep deprivation, a previous literature review indicates a 22% reduction in task accuracy during nighttime and a 7% percent reduction during daytime hours, further emphasizing the severity of this issue (Wickens et al., 2015).

In addition, endogenous and exogenous attention is equally impacted in the latter phases of attention processing under conditions of total sleep deprivation, as research indicates. This suggests an increased likelihood of errors when tasks requiring prolonged attention are performed while totally sleep-deprived (Trujillo et al., 2009). Moreover, reports from military officers have illustrated that total sleep deprivation can lead to more severe outcomes, such as frequent hallucinations that may appear during extended periods without sleep (Pallesen et al., 2018).

Total sleep deprivation, over a period of 24 to 36 hours, not only significantly worsens acute anxiety and the stress reaction, but it also reduces the capacity to manage emotions, with these adverse effects becoming progressively worse (Pires et al., 2016). Additional adverse effects were investigated by studies involving active duty military personnel enduring 40 hours of total sleep deprivation, suggesting a decline in several executive functions such as inhibitory control, attention, and switching tasks (Aidman et al., 2019). Likewise, a related study indicated a significant worsening in moral judgment, particularly in emotionally intense scenarios, after these personnel experienced more than 48 hours of total sleep deprivation (Killgore et al., 2007). Also, it has been shown that total sleep deprivation did not affect the number of personal moral scenarios deemed appropriate by individuals with high emotional intelligence. However, for those with normal emotional intelligence, an increased tendency to judge personal moral scenarios as appropriate was associated with total sleep deprivation (Killgore et al., 2007). These findings suggest that the central abilities crucial for decision-making in the military may be impaired by total sleep deprivation, with potentially even more significant impacts on individuals possessing normal emotional intelligence.

Previous research has shown that total sleep deprivation may also negatively impair oral-auditory communication, memory, and self-control and increase the chances of occurrence of risky behaviour (Christian & Ellis, 2011; Kim et al., 2001). Therefore, lack of misunderstanding in giving or obtaining commands, memorizing service duties, and the potential presence of risks for inappropriate behaviour may possibly appear in sleep-deprived soldiers (United States, Department of Defense, 2021). Previous studies have also revealed that total sleep deprivation can lead to a decrease in blood flow velocity in the middle cerebral artery (Csipo et al., 2021). This reduction may cause various cognitive and behavioral issues, such as fatigue, diminished sustained attention, and slowed reaction time (Csipo et al., 2021). In a military setting, where performance demands are exceedingly high, another crucial factor that could determine the difference between life and death is the reaction time of a soldier (Yanovich et al., 2015). Previous research has shown increased reaction times across different tasks in sleep-deprived medical professionals following a night shift (Saadat et al., 2017). This pattern of decreased reaction time was similarly shown in military personnel who had undergone two days of total sleep deprivation (McLellan, 2005).

The decrement in reaction time due to total sleep deprivation could cause detrimental consequences on related task performance, notably in light of existing research that highlights a significant correlation between marksmanship performance and reaction time (Kelley et al., 2011). Based on that, it may be essential, given the crucial role of a soldier's capacity to quickly and accurately discharge their weapon; such abilities can indeed exert a determinative influence on the probability of becoming a casualty or not.

Based on a previous systematic review study (Petrofsky et al., 2021), few studies demonstrated the exact impact of total sleep deprivation on shooting accuracy. A significant decline in both the speed and accuracy of marksmanship has been documented following 72 hours of total sleep deprivation (Tharion et al., 2003) and also by reductions in the accuracy of marksmanship, reaching an aggregate decline of 61% after a 48 hours of total sleep deprivation in military personnel (McLellan, Kamimori, Bell, et al., 2005).

In addition to total sleep deprivation's detrimental effects on marksmanship speed and accuracy, research has also shown impairments in stimulus differentiation among military cadets after 24 hours of total sleep deprivation (Maddox et al., 2009). This phenomenon may eventually result in misunderstanding military duty reports or misidentifying a fellow soldier, which can have fatal consequences under combat conditions (Yarnell & Deuster, 2016). Concerning that, a previous study investigated the impacts of sleep deprivation on marksmanship performance (using a friend-foe differentiation test) without interventions by other disturbing stress conditions (C. D. Smith et al., 2019). The identification of friendly targets was observed to be more precise in situations demanding a lower cognitive load, whereas an increase in errors occurred under conditions requiring a higher cognitive load (C. D. Smith et al., 2019). Moreover, it was noted that with the progression of sleep deprivation (3, 20, 44 and 68 hours), there was an 8% deceleration in soldiers' trigger pull response time to engage a foe across each test period relative to the initial testing period (C. D. Smith et al., 2019).

In conclusion, survey data revealed that almost 15% of soldiers acknowledged their participation in accidents is deeply troubling, especially considering that sleep deprivation was identified as a contributory element by half of these respondents (LoPresti et al., 2016). Although the available literature exploring this correlation is limited, some estimates suggest that cognitive fatigue caused by total sleep deprivation could be responsible for up to 20% of combat casualties, such as friendly fire incidents (Rasmussen, 2007; Wilson et al., 2015).

Thus, highlighting the importance of maintaining proper sleep and efforts to avoid or minimize total sleep deprivation to ensure optimal military performance and safety.

b) Effects of total sleep deprivation on physical abilities

Previous research on the impact of different sleep deprivation protocols on physical performance in the general population of night shift workers (Jay et al., 2015) or professional sportsmen (Fullagar et al., 2015; Halson, 2014) is relatively comprehensive. However, understanding this within the military sector remains relatively limited, probably because of the difficulty of mimicking battlefield conditions during testing and the complexity involved in defining military physical performance (Grandou et al., 2019). To effectively fulfil the military service demands (sprinting across the battlefield, carrying heavy loads, or dragging wounded fellows to safety etc.) (Kraemer & Szivak, 2012) it is crucial that a soldier needs to maintain a high level of physical readiness, be strong, explosive and have excellent endurance capacity (Friedl et al., 2015).

Prolonged physical activities like loaded ruck marches are common military tasks (Foulis et al., 2017). As stated in a previous study, soldiers who could maintain their aerobic fitness during total sleep deprivation and extended physical exertion may perform better in such military tasks (Friedl et al., 2015). Nonetheless, comparing these studies may be challenging due to different sleep loss protocols and other factors playing a role (i.e. motivation, training or diet) (Grandou et al., 2019).

Previous studies investigating the effect of total sleep deprivation on time to complete aerobic physical tasks and physiological reactions have shown mixed results (Grandou et al., 2019). No significant changes were observed in completion time for a 1.6 km walk (Tomczak et al., 2017) and progressive cycle ergometer test (Vaara et al., 2018) following 36 and 60 hours of total sleep deprivation, respectively. However, the heart rate (by 11%) and maximal blood lactate (by 24%) at the completion of these two tests were notably lower. Nonetheless, it remains a matter of debate whether these modified responses, which do not cause any change in time to exhaustion, correlate with performance (Vaara et al., 2018).

It appears that submaximal and maximal aerobic performance is relatively unaffected by total sleep deprivation, altering physiological responses possibly due to changes in glycogen metabolism (Grandou et al., 2019). Interestingly, the interplay between partial sleep deprivation and energy intake on aerobic performance is also relevant, indicating that energy deficits may possibly reduce aerobic capacity more than sleep loss alone (Grandou et al., 2019). However, research was not established on total sleep deprivation protocols yet. Thus, future research needs to control for these variables to understand better the total sleep deprivation impact on military personnel's aerobic capacity.

The subsequent physical ability necessitating emphasis within the military is anaerobic capacity, specifically in the lower body (Friedl et al., 2015). Interestingly, the academic literature seems to lack investigations specifically examining the effects of total sleep deprivation on soldiers’ upper body's anaerobic capacity (Grandou et al., 2019). It is essential to acknowledge that the previous observation pertains to the current state of research and may evolve. Future research contributions could significantly augment our understanding of this domain. In the face of prolonged sleep deprivation, the preservation of the anaerobic capacity of the lower body has vital importance for military personnel (Friedl et al., 2015). This is highlighted by the frequent requirement in both training and combat scenarios for quick manipulation of loads or sprinting, thereby placing significant demands on lower-body anaerobic capabilities (Friedl et al., 2015).

Analyzing prior research reveals that total sleep deprivation can negatively influence lower body anaerobic performance (Tomczak, 2015; Tomczak et al., 2017). There were reported military pilots' performance declines in the series of maximal-effort sprint tests (Tomczak, 2015) and air force cadets (Tomczak et al., 2017) following 36 hours of total sleep deprivation. Notably, a significant decrease was seen in 15 meters sprint (2-8%) and squat sprint speeds tests (10%). Additionally, a short recovery period of 7-8 hours of sleep did not fully restore performance, indicating a longer recovery time might be needed to recover lower body anaerobic capacity after prolonged total sleep deprivation (Tomczak, 2015; Tomczak et al., 2017). While these findings contribute to our understanding of total sleep deprivation and lower body anaerobic performance, questions remain. The relevance of sprint performance for members of the air force and pilots, for instance, may be a subject of debate (Grandou et al., 2019). In light of this, future research aiming to refine our understanding should take into account confounding factors, such as added load or physical condition, and also adding familiarization sessions would be highly recommended (Grandou et al., 2019). Implementing such measures would be essential to gain a more comprehensive understanding of how total sleep deprivation affects lower body anaerobic performance, particularly in relation to tasks frequently performed by military personnel (Grandou et al., 2019).

Considering the previous narrative review (Grandou et al., 2019), the subsequent crucial physical ability required for the efficient execution of routine military duties like causality drag, loaded sprinting and carries or throwing is upper and lower-body muscular strength (Kraemer & Szivak, 2012). Primarily due to its direct influence on battlefield performance, the recognition of strength as a fundamental element of soldiers' fitness is progressively increasing (Kraemer & Szivak, 2012). Thus, maintaining muscular strength during periods of total sleep deprivation is of utmost importance for sustaining effectiveness in military combat operations (Kraemer & Szivak, 2012). For those reasons, the handgrip strength and maximal isometric knee extension force (Vaara et al., 2018) tests are frequently utilized in military research as a significant predictor for assessing muscular strength due to their potential implications for enhanced military performance (Moraes Gonçalves et al., 2018).

However, previous research exploring the effects of total sleep deprivation on muscular strength has yielded inconsistent outcomes. For instance, it was shown that muscular strength through a handgrip strength test after 36 hours of total sleep deprivation significantly decreased in military pilots by 6.6% (Tomczak, 2015) and also naval seamen (Foo et al., 1994; How et al., 1994) after 42 hours of total sleep deprivation. After total sleep deprivation, the decrease in handgrip strength may be associated with either modification in the motor unit firing rate or a drop in the recruitment of muscle fibers (Legg & Patton, 1987).

Contrarily, a prior study (Goh et al., 2001) observed that soldiers' handgrip strength differed over a 24-hour period, irrespective of sleep conditions (i.e., whether they had full night of sleep or were totally sleep deprived), with increasing handgrip strength until 18.00 and then experiencing a consistent decrease throughout the night. Additionally, contrary to prior findings, when total sleep deprivation extended beyond 42 hours (Foo et al., 1994; How et al., 1994), an improvement in handgrip strength was observed between the 42nd and 66th hours of total sleep deprivation. However, a subsequent decline was noted from the 68th to 102nd hours of total sleep deprivation. Furthermore, it was observed that even after enduring 60 hours of total sleep deprivation, military cadets did not exhibit a significant decrease in the maximal isometric force of knee extension, electromyography or rate of force development (Vaara et al., 2018).

The potentially unaffected handgrip strength, even under conditions of total sleep deprivation, might be due to the body's ability to adapt to fatigue-induced effects resulting from lack of sleep (Grandou et al., 2019). This adaptation could stabilize or offset the impacts of total sleep deprivation. Additionally, research has indicated that muscular strength does not remain constant but oscillates following a circadian rhythm (Sargent et al., 2010), the body's internal clock that operates roughly on a 24-hour cycle. Therefore, the natural cycle of muscular strength peaks and troughs may mask or mitigate sleep loss's weakening effects on handgrip strength. Not taking into account the various confounding factors may make it appear as though total sleep deprivation does not impact handgrip strength (Grandou et al., 2019).

This literature review aimed to interpret current studies to highlight the impact of total sleep deprivation on military personnel's-related physical performance. Nevertheless, cation is required when reporting these results since the present literature does not adequately apply randomized controls every time (Grandou et al., 2019). To fully understand how total sleep deprivation affects physical abilities in military personnel, further research that effectively controls for these limitations is needed.

2.5. Ergogenic aids

As described earlier, total sleep deprivation can be a very common phenomenon in highly demanding professional fields such as the military. However, despite enduring prolonged periods of inadequate sleep or no sleep at all, soldiers are often expected to maintain optimal levels of physical and cognitive performance (Tait et al., 2022). Therefore, to counteract the potential adverse effects of total sleep deprivation and to confront these high-demand challenges, many soldiers have turned to ergogenic aids (H. R. Lieberman et al., 2010). This generally includes substances which were specifically invented to enhance physical and cognitive performance or improve recovery (H. R. Lieberman et al., 2012).

Ergogenic aids are widely used across the spectrum of sports, from amateurs to professionals (Ahrendt, 2001). Around half of the general population admits to using dietary supplements of some sort, while the prevalence of their use among athletes can range from 76 to 100% (Ahrendt, 2001). Whereby the example of the most recognized and frequently used legal ergogenic aids include substances such as caffeine, creatine, branched-chain amino acids, glutamine, β-alanine, nitrates, and hydroxymethylbutyrate (Cameron et al., 2018; Del Coso et al., 2011; Froiland et al., 2004; Jagim et al., 2016; Juhn, 2003; Kedia et al., 2014). Among these aids, caffeine stands out as one of the most frequently utilized and broadly recognized, mainly due to its stimulatory effects (H. R. Lieberman et al., 2010). Soldiers commonly report integrating it extensively into their dietary supplements, including caffeine products (i.e., caffeine pills, energy drinks, chewing gums), to boost performance, elevate energy levels, promote endurance, and assist in weight loss (H. R. Lieberman et al., 2010). These benefits align with caffeine's physical and cognitive advantages, which are often highlighted in mainstream media (H. R. Lieberman et al., 2012).

In the following section, we aim to discuss both the positive and negative effects of caffeine usage in military personnel, particularly as a response to total sleep deprivation. Additionally, we aim to explore how caffeine usage can help manage the drawbacks of sleep deprivation and consider the potential downsides, especially regarding high dosages. Soldiers, especially men, averagely consume considerably more caffeine on a daily basis, typically from 212 mg to 285 mg (Chaudhary et al., 2021; Knapik et al., 2017; H. R. Lieberman et al., 2012), compared to the average U.S. general population, whose intake typically lies between 165 mg and 210 mg per day (Fulgoni et al., 2015). Based on previous research (Guest et al., 2021), the suggested intake for achieving performance-enhancing effects from caffeine is 3 to 6 mg per 1 kg of body weight, while health impact assessments indicate that daily caffeine consumption of about 400 mg is generally considered as a safe dose (Temple et al., 2017).

Considering the fact that specific components of military food packages (such as meals-ready-to-eat) containing caffeine products are broadly accessible (H. R. Lieberman et al., 2012), the military provides guidelines to soldiers for a maximum caffeine intake per day, ranging from 400 mg to 1000 mg, whilst also cautioning about the potential side effects that may arise if this maximum dose is exceeded (Committee on Military Nutrition Research, 2001).

Based on a previous literature review, consuming moderate amounts of caffeine, around 300 mg, may help to sustain attention, alertness, and vigilance in sleep-deprived individuals (Crawford et al., 2017). For instance, research conducted among military personnel indicated that caffeine (300 mg) effectively enhances performance in reaction times, attention, and executive function tests during total sleep deprivation periods of 48 to 64 hours without adversely impacting the subsequent recovery sleep (Beaumont et al., 2001, 2005; United States, Department of Defense, 2021). Additionally, studies conducted on military special forces during field training found that caffeine doses of 200 mg effectively sustained alertness during night-time surveillance tasks. Nonetheless, caffeine's influence was not found to affect any speed or accuracy of marksmanship tasks (Kamimori et al., 2015; McLellan et al., 2007; McLellan, Kamimori, Voss, et al., 2005; Tikuisis et al., 2004).

Furthermore, research conducted with U.S. Navy Sea-Air-Land candidates after exposure to 72 hours of total sleep deprivation demonstrated significant improvements in vigilance, reaction time, and alertness with the administration of 200 mg and 300 mg doses of caffeine. However, these doses did not result in an enhancement of marksmanship skills (H. Lieberman et al., 2002). In another study involving military pilots, a total caffeine intake of 400 mg helped to mitigate cognitive task performance decrements during 9 hours of total sleep deprivation (Doan et al., 2006). Contrarily, in the following research, a single dose of 200 mg of caffeine was insufficient to reverse the decline in simulator performance (Lohi et al., 2007) and vigilance test (Kilpeläinen et al., 2010) among military pilots after an extended total sleep deprivation period of 37 hours.

Transitioning from cognitive benefits, caffeine's impact on physical function also warrants exploration. Multiple studies have been conducted to investigate the impact of caffeine usage on sports performance (Burke, 2008; Mielgo-Ayuso et al., 2019), providing a plethora of evidence in this area. Nevertheless, research specifically addressing the effects of caffeine on the physical performance of total sleep-deprived military personnel is comparatively sparse (McLellan et al., 2016).

However, previous research, both in the laboratory and field settings, has shown the positive effects of caffeine usage on physical performance under stressful conditions, typically simulating sustained military operations (containing sleep deprivation) (McLellan et al., 2016). Whereby, a total caffeine dose from 200 mg to 600 mg has been found to improve physical performance indicators, such as time-to-exhaustion during treadmill runs and efficiency in a sandbag piling task , short sprints speed, muscle strength and endurance (McLellan et al., 2016), and improved target engagement time during marksmanship performance (Tikuisis et al., 2004). Further research with soldiers from special units revealed caffeine's effectiveness in improving 6.3 km run times when a 200 mg dose was administered roughly an hour before the run (McLellan, Kamimori, Voss, et al., 2005). Following study investigating the effects of caffeine during repeated nights of sustained wakefulness followed by restricted daytime sleep demonstrated caffeine's capability to maintain physical performance during obstacle course tests (Kamimori et al., 2015).

In conclusion, although caffeine and other stimulants may offer temporary cognitive and physical performance enhancement effects, enabling users to delay but not eliminate the need for sleep eventually (Wesensten et al., 2011). It is important to note that high doses of caffeine can subsequently interfere with the recovery sleep that is vital after experiencing total sleep deprivation (LaJambe et al., 2005). Additionally, inappropriate caffeine use in military settings, such as consuming them near bedtime, can interfere with regular sleep cycles and quality of sleep. This could lead to a higher daytime dependency on caffeine to remain alert, thereby establishing a harmful cycle where increased daytime caffeine consumption is followed by disrupted sleep caused by caffeine itself (Good et al., 2020).

Although the effects of caffeine can last several hours, its onset is generally noted several minutes after consumption due to the required digestion time (Graham, 2001). This factor may restrict its handiness for immediate cognitive or performance enhancement. As a result, ergogenic aids with a faster onset of action could be potentially more beneficial in situations that require prompt arousal of physical or cognitive performance in soldiers. Therefore, our initial study aims to delve deeper into the possibility of one such fast-acting ergogenic aid: ammonium inhalants.

3. The rationale for the first study

Both realms of competitive sports and military service are represented by a relentless pursuit of excellent performance with minimum mistakes, where even slight enhancements can make a substantial difference (Klymovych et al., 2020). Based on that, it is logical that numerous athletes and soldiers alike rely on various supplements and ergogenic aids for physical and psychological performance enhancement (Maughan et al., 2007; Pritchard et al., 2014). As such, using nutritional supplements and ergogenic aids has become a daily habit (Bishop, 2010; Del Coso et al., 2011; Froiland et al., 2004; Maughan et al., 2007). Specifically, substances such as caffeine, one of the most famous and used ergogenic aid (H. R. Lieberman et al., 2010), and also other less known ergogenic substances, including β-alanine, nitrates, branched-chain amino acids, glutamine, taurine and others (Cameron et al., 2018; Del Coso et al., 2011; Froiland et al., 2004; Jagim et al., 2016; Juhn, 2003; Kedia et al., 2014). However, gaining any positive effects of those ergogenic aids takes a certain amount of time, typically at least 10 minutes (Gonzalez et al., 2011), from consumption to manifest, which may not be sufficient in situations demanding immediate alertness or performance enhancement. While manageable in organized professional sports and contests, this time lag can severely impede or endanger personnel in a military environment.

Unlike athletes, soldiers often find themselves in life-threatening situations where split-second decisions and immediate action may be a matter of life and death, thus waiting 10 or more minutes for an ergogenic aid to take effect is simply not practical. This critical need for rapid response in high-stakes situations drives the quest for finding fast-acting performance enhancers (Harman et al., 2008). Consequently, soldiers and their commanders may actively be exploring these aids, seeking solutions that can rapidly enhance their operational readiness and performance under challenging conditions (Harman et al., 2008).

Fast-acting ergogenic aids like ammonia inhalants have gained attention in this context, being used as rapid-acting stimulants to improve vigilance and short-term high-intensity performance (McCrory, 2006). Despite their high prevalence among athletes (Herrick & Herrick, 1983; Prewitt, 2016; Pritchard et al., 2014; Rivera-Brown & Frontera, 2012), the current understanding of these fast-acting ergogenic aids is limited and inconclusive.

Therefore, the first study titled *"Effects of Ammonia Inhalants in Humans: A Review of the Current Literature Regarding the Benefits, Risks, and Efficacy"* (Malecek & Tufano, 2021) sought to comprehensively explore and consolidate the existing literature on ergogenic effects of ammonia inhalants, assessing their benefits, risks, and physiological effects, thereby providing a foundation for further research in this area.

The resulting manuscript was published in 2021 in the Strength & Conditioning Journal. The substantive content of the text remains untouched. However, to ensure seamless integration and uniformity within the entire dissertation thesis document, minor changes have been made to the original formatting of the submitted manuscript. The changes implemented are confined to the citation style and table layout, which have been adapted to meet the format specifications of this dissertation thesis document. Furthermore, the actual references have been restructured and can be found enumerated at the end of this dissertation thesis document.

4. Effects of Ammonia Inhalants in Humans: A Review of the Current Literature Regarding the Benefits, Risks, and Efficacy

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List of abbreviations

Abbreviation	**Definition**
AI	Ammonia inhalants
ppm	Parts per million
WADA	World Anti-Doping Agency
$PetCO_2$	Partial pressure of end-tidal CO_2
HR	Heart rate
MAP	Mean arterial pressure
MTP	Mid-thigh pull
MCAv	Middle cerebral artery blood flow velocity
BR	Breathing rate
FEV1.0	One-second forced expiratory volume
FVC	Forced vital capacity
AMRAP	As many repetitions as possible
BS	Back squat
1RM	1-repetition maximum
BP	Bench press
RFD	Rate of force development
pRFD	Peak of force development
EMG	Surface electromyography
IMTP	Maximal-effort isometric mid-thigh pull
CMJP	Bodyweight countermovement jump power
CMJ	Bodyweight countermovement jumps
BJ	Broad jump
MP	Anaerobic mean power
PP	Anaerobic peak power
SAFG	Simulated American football game
VL	Vertical leap
VVR	Vicks VapoRub

Abstract:

Ammonium inhalants (AIs) are used to improve athletic performance, but their use has preceded the research process. Oftentimes, strength-based athletes use AIs to postpone acute fatigue or increase arousal. Despite the widespread use of AIs, the amount of research examining its physiological effects, efficacy, and safety is astonishingly low compared to other ergogenic aids that have been extensively researched. Therefore, the purpose of this review is to provide sports science researchers, strength and conditioning professionals, medical professionals, and other practitioners with the most up to date information about the benefits, risks, and physiological effects of AIs. To date, there is a lack of evidence to support anecdotal claims of increased cognitive arousal and greater strength performance. However, there may be a short-term effect of AIs on the cardiorespiratory system (possibly increasing breathing rate and heart rate approximately 15 to 30 seconds), but further research is needed to support these findings and to determine how the short-term cardiorespiratory effects may affect other physiological and performance measures. Lastly, although evidence does not indicate that AIs are dangerous in healthy populations, sport and health professionals should be aware of the potential risks of AIs to prevent any unlikely, but possible, difficulties.

Key Words: Ammonia inhalation, Ergogenic aids, Smelling salts, Strength, Heart rate, Power

4.1. Introduction

Considering the importance of physiological arousal before competition (D. Perkins et al., 2001; Tod et al., 2003), it stands to reason that many athletes look to various supplements and ergogenic aids to help immediately improve performance (Maughan et al., 2007; Pritchard et al., 2014). Therefore, the daily use of nutritional supplements and ergogenic aids has become an everyday necessity for many athletes (Bishop, 2010; Del Coso et al., 2011; Froiland et al., 2004; Maughan et al., 2007). In particular, caffeine and other ergogenic pre-workout components such as branched-chain amino acids, taurine, glutamine, nitrates, creatine or β-alanine tend to be the most prevalent amongst athletes during both training and competition to improve acute exercise performance (Cameron et al., 2018; Del Coso et al., 2011; Froiland et al., 2004; Jagim et al., 2016; Kedia et al., 2014). However, any physiological or cognitive effects of these compounds is postponed, as they must be digested, absorbed, and distributed throughout the body. Accordingly, pre-workout supplementation often occurs anywhere from 10 (Gonzalez et al., 2011) to 60 minutes (Graham, 2001) before a performance. In some instances, athletes desire a more rapid form of psychological or physiological arousal. Therefore, the inhalation of ammonia inhalants (AI) commonly serves as a fast-acting "pre-workout stimulant" whereby athletes hope to rapidly improve performance during both training and competition (Herrick & Herrick, 1983; McCrory, 2006). Despite their widespread use, the benefits, risks, and efficacy of AIs are quite unknown. Therefore, the purpose of this review is to summarize the available literature on AIs and performance, while also highlighting the need for future research in the area.

Within this paper, the history and composition of AIs are briefly explained, and the general responses to AI inhalation are summarized. The possible risks of AI inhalation are introduced, and the modern use of AIs is explained. As the focus of this paper is on the benefits, risks, and efficacy of AI use in humans (specifically human performance), a review of literature was conducted, and each study is described in detail.

For the purpose of this literature review, a search of the following electronic databases by the author JM was performed: MEDLINE by PubMed, SCOPUS, Web of Science, and Google Scholar. Due to the paucity of research on the topic, search terms were quite broad and included a variety of terms including: ammonia, inhalation, performance, and smelling salts. Additionally, due to the lack of peer-reviewed articles, a manual search from the reference list of all retained articles was performed until April

2020. Despite these efforts and generous search criteria, the search procedure only resulted in a total of 4 peer-reviewed studies (Bartolomei et al., 2018; Perry et al., 2016; Richmond et al., 2014; Vigil et al., 2018). Therefore, to increase the breadth of this review, we also included 6 conference abstracts (Archer et al., 2017; McEvoy et al., 2019; Mitchell et al., 2015; Rivera et al., 2017; Secrest et al., 2015; Witherbee et al., 2013) and 1 master's thesis (Groop, 2019).

4.1.1. Ammonia inhalant background and composition

Chemically, AI compounds of ammonium carbonate are colorless-to-white, crystalline solids ($(NH_4)_2CO_3$). Although ammonium carbonate can be mixed with products such as eucalyptus, lemon oil, or lavender oil (Medicines, 2021) most of the widely used AIs are mixed with water and ethanol, resulting in labels that indicate "aromatic spirits of ammonia" since the final product is no longer pure ammonia (McCrory, 2006). Dating back to nearly the 13th century, AIs (known as smelling salts, sal volatile, or spirit of hartshorn) were widely used to treat lightheadedness, dizziness, and fainting. Using AIs for these purposes continued during the times of Victorian Britain where AIs were regularly carried in decorative boxes which contained a sponge soaked in an AI mixture with perfume in vinegar or alcohol (BBC News, 2020; Friedman, 2020; McCrory, 2006). The use of AIs continued and was highly recommended by the British Red Cross and St. John Ambulance during the Second World War (First Aid Kits, 2020), resulting in American soldiers carrying AIs on a medical belt for front line first aid as a stimulant in situations like fainting after haemorrhage or shock (Wever et al., 2016). Moving forward to today, the only allowed use of AIs in the United States is for the general population and only for first aid purposes such as treatment of fainting or dizziness (Drugs, 2020; Velasquez, 2011). However, AIs are widely used in various sport settings, meaning that their effects should be better understood, warranting in-depth investigation.

Today, AIs can be obtained in different forms, most commonly as liquid, or capsules. Of these, capsules are the most common and contain, based on weight, 15 to 20% of ammonia and 30 to 40% of ethyl alcohol, which is approximately 0.33 ml of each component (Dynarex, 2020; Safety Data Sheet, Ammonia Inhalant Solution, 2020). Although, capsules are generally the most used form in most team sports, capsules, powder, and liquid forms are often used in powerlifting (ESPN, 2017). Based on previous research (McCrory, 2006) and the safety data sheets for commercially available AIs, it is recommended to hold and inhale the substance 10 to 15 cm away from nostrils to achieve the desired arousal response. Despite these widely used guidelines, the physiological responses to AI inhalation is not fully understood.

4.1.2. Response to inhalation

Although the inhalation of commercially available AIs is prevalent in different sport settings (Perry et al., 2016), the physiological responses to AIs inhalation remain largely unknown. Nevertheless, some general observations have been made. Firstly, since ammonia is water soluble, AIs inhalation may be associated with irritation of the upper respiratory tract through the mucosa, and consequently, may result in lung irritation which results in coughing and a potential increased respiratory rate (Widdicombe & Lee, 2001). Secondly, due to the possible irritation of the respiratory passages, the sympathetic nervous system may respond as part of the autonomic nervous system (Velasquez, 2011). The autonomic stimulations are presumably associated with the activation of the olfactory and trigeminal nerves (Bensafi, 2002), which can subsequently result in activating the adrenergic receptors in peripheral tissue, releasing norepinephrine and increasing cardiac output and respiratory rate (Marshall, 1982). Although this sequence of events is logical, the specific moments when the respiratory rate increases and the sympathetic nervous system is stimulated are unknown. Considering the purpose of AI use and these underlying mechanisms, it can be hypothesized that AI inhalation could result in performance-enhancing effects, but the physiological reaction and timing remain unclear (Ott & Vilstrup, 2014; Perry et al., 2016; Velasquez, 2011).

The majority of research has reported that inhaling AIs results in what can be described as a “psyching-up” or “pick me up” effect (McCrory, 2006; Tod et al., 2003; Velasquez, 2011). It is likely that this effect is the result of altered fundamental breathing patterns as the stimulated respiration muscles operate faster, increasing the respiratory rate, and possibly resulting in a higher level of vigilance (McCrory, 2006; Pritchard et al., 2014; Vigil et al., 2018). However, to prove these claims, the current literature contains an inadequate amount of empirical evidence (Perry et al., 2016).

Considering the lack of research investigating the effects of AIs, some authors (Perry et al., 2016) have tried to decipher its effects by noting the partial resemblance between the immediate effect of AIs inhalation and delayed effect of caffeine supplementation. Regardless of whether the mechanisms of caffeine consumption and AIs inhalation are similar, the subsequent physiological reaction after AIs inhalation could release methylxanthine and norepinephrine, possibly enhancing vigilance, increasing arousal, and decreasing exhaustion. Although these effects seem similar to the purported effects of caffeine, caffeine consumption can also have adverse effects such as gastrointestinal discomfort, anxiety, inability to concentrate, insomnia, and cardiac arrhythmia (Silver, 2001). Although those responses represent the possible acute effects of caffeine intake (Nehlig et al., 1992; Perry et al., 2016), whether the adverse or positive effects linked with caffeine consumption are consistent with those after AIs inhalation remains unclear, and future research is needed.

4.1.3. Possible risks of inhalation

Respiratory stimulants such as AIs are mostly used for arousing consciousness by way of ammonia (NH_3), which can be toxic. Even though ammonia is commonly used as a chemical component of pesticides, fertilizers, and household cleaners, there are some potential risks of exposure to ammonia vapour (Leduc et al., 1992), which, in short, depend on the vapour concentration, exposure time, and amount of inhaled vapours (Leduc et al., 1992).

Based on previous animal research and human case studies (Herrick & Herrick, 1983; Leduc et al., 1992; D. Perkins et al., 2001) exposure to large doses (more than 1,500 ppm for 30 minutes) of concentrated ammonia vapours (Pritchard, 2007) may result in injuries such as allergic reactions, respiratory airway burns and difficulties, oedema, and distress. Moreover, it can negatively affect the whole respiratory system, can cause a variety of acute damaging respiratory and neurological conditions, and in the worst cases, can be fatal (Perry et al., 2016; Pritchard, 2007). Whereas a single case of death occurred after exposure to 10,000 ppm of ammonia for an unspecified time amount (Pritchard, 2007), another less severe instance occurred where a single exposure to 20,000 ppm of ammonia resulted in lung collapse and body weight loss (M. Perkins et al., 2017). Therefore, it appears as though high doses of ammonium lead to severe, but individual responses. Nevertheless, commercially available AIs inhalants capsules release about 50 to 100 ppm of ammonia vapours, which is significantly lower than the dangerous amounts that have been previously cited to cause serious injury.

Aside from serious consequences in a select few cases, another potential risk of using AIs is allergic reactions that may require medical attention. Presumably using a low-dose commercially available AI, another case study described an allergic reaction to a single acute use of AI during a powerlifting competition (Herrick & Herrick, 1983).

Although the athlete was a regular user of AIs, after inhaling an AI from a capsule followed by attempt for setting a national record, the female powerlifter started to experience rhinorrhoea, rhinitis, conjunctivitis, dizziness, and a severe headache. These progressively became more serious and within an hour, wheezing, shortness of breath, periorbital swelling, and a loss of vision occurred, lasting for up to an hour. The symptoms were then resolved immediately via medical treatment, but the episode is worth noting because it occurred in an athlete who regularly exposed herself to AIs. Although AI producers mark their packages with a warning where they advise not to use if the patient has a flushed face, there is no other warning regarding the presence of hypertension or possible allergic reaction for some ingredients (Herrick & Herrick, 1983). In conclusion, increased caution should be used before and after AI inhalation. Furthermore, the particular ingredients in each AI product should be cross-referenced with all known allergies for anyone who may come into contact with it (Groop, 2019).

Not only are AIs used by seemingly healthy athletes before performing a single bout of maximal effort (e.g., powerlifting), but they are often used in contact sports such as rugby, ice hockey, American football, and others where head injuries may be present. Naturally, a certain degree of relative risk may appear when using AIs following contact-induced head injuries such as concussions. If AIs are used right after experiencing unconsciousness or dizziness after head contact, the concussive symptoms may be masked, and the diagnosis of the concussion may be complicated. In these cases, it is possible that masking head injury symptoms may lead to continued participation in the competition, which could worsen the consequences of the head injury (Velasquez, 2011). Furthermore, as described earlier, the rapid and extreme nose and lung irritation combined with an instant withdrawal reflex may cause subsequent involuntary inhalation following expeditious and sudden movements that can result in additional unwanted brain injuries of concussed athletes (McCrory, 2006). However, just as there is a lack of evidence to support the use of AIs following possible head injuries, there is also a lack of evidence negating its use for this purpose. Therefore, future research, although difficult and perhaps ethically questionable, should aim to determine the effects of AIs in response to real-world use during contact sports.

Lastly, because of the potential lung irritation, people with asthma and respiratory problems should be aware that the inhalation of AIs may result in acute breathing difficulty (Herrick & Herrick, 1983; Velasquez, 2011). Furthermore, because of oscillations in heart rate, blood pressure, and peripheral blood flow, the individual risk of syncope can occur (Julu et al., 2003). In some cases, increased tearing production or coughing may occur as well (Inchem, 1986). Thus, athletes or persons with respiratory problems may want to avoid using AIs, especially when not under proper medical supervision.

In conclusion, AI inhalation is legal in the United States for reviving fainted persons but necessarily not for improving athletic performance (albeit not specifically illegal, either). Therefore using AIs for anything other than its approved uses requires increased caution (Velasquez, 2011). Additionally, based on previous research (Herrick & Herrick, 1983), the application of anything that can be potentially misused by athletes or can have adverse effects unquestionably needs to be monitored by strength and conditioning professionals and medical staff. Therefore, basic knowledge and training should be provided for sport performance personnel, and they should maintain a certain level of knowledge to be experienced in the case of an emergency (Velasquez, 2011). Nevertheless, by understanding these procedures and following safety precautions on the product's safety sheets during inhalation of AIs, it seems to be unlikely that any permanent or severe consequences could spur from acute inhalation in healthy athletes (Gorguner & Akgun, 2010).

4.1.4. Ammonia inhalants nowadays

The use of AIs by athletes, especially those in strength-based sports such as weightlifting, powerlifting, discus and hammer throwing, shot-putting, ice-hockey, rugby, and American football, is a widespread phenomenon (Herrick & Herrick, 1983; Prewitt, 2016; Rivera-Brown & Frontera, 2012). Furthermore, within an international survey of powerlifters competing in the International Powerlifting Federation (IPF), 130 out of 256 lifters reported inhalation of AIs during competition. Accordingly, the most used were AIs inhalation prior to deadlifts (compared to back squat and bench press), which is the last discipline of powerlifting competitions (Pritchard et al., 2014). Moreover, lifters reported feelings of reduced tiredness and fatigue after inhalation of AIs and, hence, it may lead to enhance mental concentration which can be crucial during the last lifts of powerlifting competition (Pritchard et al., 2014). Athletes also state that inhaling AIs helps to create an alert state of mind, which supports succeeding in challenging tasks or at improving performance with less effort. Nonetheless, previous researchers (McCrory, 2006; Perry et al., 2016; Pritchard et al., 2014; Velasquez, 2011) have stated that there is a lack of evidence to confirm these anecdotal benefits, which are described in further detail below.

It is evident that the inhalation of AIs is becoming more relevant and common among athletes (McCrory, 2006), which means that competition rules must acknowledge its use and create rules about AI inhalation. Although AI inhalation is not currently listed in the World Anti-Doping Agency (WADA) list of prohibited substances and methods, lifters taking part in IPF powerlifting competitions are not allowed to inhale AIs directly in front of an audience (International Powerlifting Federation, 2020; World Anti-Doping Agency, 2020). Therefore, it is interesting that ethical reasons ban its visible use in some settings, but other sports have not taken similar actions, or simply banned its use altogether. Likely, a lack of research on the effects and risks of AIs does not allow sporting bodies to move forward in either approving or banning AI use. Therefore, further research should be conducted in this area in order to confidently support or refute its use.

4.1.5. Researched effects on the cardiorespiratory system

Little research has been done to determine the acute effects of AIs on the cardiorespiratory system. For instance, one study (Perry et al., 2016) investigated the physiological effects of AIs inhalation on the cerebrovascular and cardiorespiratory systems in association with anaerobic performance in 15 healthy males. Two visits were conducted. During the first visit, data were collected before and after AI inhalation to assess beat-to-beat middle cerebral artery blood flow velocity (MCAv), the partial pressure of end-tidal CO2 (PetCO2), heart rate (HR), and mean arterial pressure (MAP). During the second visit, subjects performed a maximal-effort mid-thigh pull (MTP) at various time points (immediately, 15, 30 and 60 seconds) following either AI inhalation or no inhalation with MCAv, PetCO2, HR, and MAP remaining monitored. Although HR and MCAv significantly increased 15 seconds after AI inhalation, there were no differences for any other variable at any time point. Therefore, the immediate increase in HR combined with the immediate increase of MCAv and no change in MAP indicates that transient cerebrovascular vasodilation occurred in response to AI inhalation but did not last longer than 15 to 30 seconds.

Another study (Groop, 2019) observed the sympathetic cardiorespiratory system response by assessing changes in HR and breathing rate (BR) in response to AI inhalation in 16 male ice-hockey players. HR and BR were measured for 5 seconds before and up to 60 seconds after inhalation of an AI capsule. After 6 minutes of physical rest (the time needed to complete cognitive tests, explained in the following section), subjects completed a 30-second maximal effort Wingate test on a cycle ergometer. Although there was a significant increase in HR and BR following AI inhalation, there were no ergogenic effects observed on any other variable. Moreover, the HR was the most elevated at 7.5 and 10 seconds after AI inhalation, which corresponds to the results of the previous study (Perry et al., 2016).

Furthermore, a conference paper (McEvoy et al., 2019) investigated the acute effect of AI inhalation on the respiratory system function in 22 college-aged students (11 males and 11 females). Subjects performed one-second forced expiratory volume (FEV1.0) and forced vital capacity (FVC) tests before and after AI inhalation, but there were no significant differences in any tested variables.

In conclusion, 2 studies (Groop, 2019; Perry et al., 2016) observed a significant increase in HR up to 15 seconds after inhalation. However, HR decreased back to baseline within 15 to 30 seconds (Perry et al., 2016). Although the same pattern of increase was observed in BR (Groop, 2019) no other respiratory changes were statistically significant (McEvoy et al., 2019). Based on these results, the effect of AIs on the cardiorespiratory system tends to be short term and may not serve as an ergogenic aid for performance lasting longer than 15 seconds from the moment of inhalation. Nevertheless, since MCAv is regularly used as a predictor for cognitive impairment (Bertsch et al., 2009) and considering the positive correlation between HR and cognitive performance (Luft et al., 2009), the potential increment in these variables after AI inhalation may result in increased cognitive performance (Harris et al., 2018).

4.1.6. Researched effects on cognitive function

In general, cognitive function includes multiple mental abilities such as learning, reasoning, thinking, remembering, decision making, or attention (Borson, 2010). Due to these mental abilities athletes may recognize and obtain environmental information to combine them with existing knowledge, which can play a significant role in competitive sports (Marteniuk, 1976). Although research supports the concept of increased arousal and improved cognition (Parfitt et al., 1995; Santos et al., 2014), there is still a lack of knowledge about the acute effect of AIs inhalation on cognitive functions.

For example, one study investigated the effect of AI inhalation on cognitive performance by simple and choice reaction time using a computer and basic keyboard tasks in 16 male ice-hockey players (Groop, 2019). They also tested responses to the Wingate test (explained in the following section). Cognitive tests were conducted 60 seconds after AI inhalation (but before the Wingate test) and lasted for approximately 5 minutes. There were no significant changes observed in any variables of reaction time test responses after AI inhalation compared to the same tests performed after no AI inhalation. Based on previous findings (Groop, 2019; Perry et al., 2016), the cardiorespiratory responses returned to normal 15 to 30 seconds after AI inhalation, so it is logical that no cognitive effects were present during the reaction time tests that started 60 seconds after AI inhalation. Therefore, any sympathetic arousal that may have been present right after AI inhalation may have already disappeared by the time the cognitive tests began.

Although the literature investigating the effects of AI on cognition is scant, anecdotal evidence (supported by the wide-spread presence of the "psyching up effect") indicates that AIs may in fact have a cognitive effect, but the effect just has not been fully elucidated. Therefore, future research on the topic is needed and could include different AI timing, different cognitive tests, different populations, and the like (McCrory, 2006). Furthermore, athletes likely desire the purported psyching up effect not only to increase cognitive arousal or vigilance, but to harness that cognitive effect to improve strength or power performance.

4.1.7. Researched effects on strength and power

Presumably, since many athletes believe that increasing arousal before a competition can enhance muscle strength and power performance, using AIs as an ergogenic aid has become widespread amongst athletes (Pritchard et al., 2014; Tod et al., 2003). Even though a survey (Pritchard et al., 2014) indicated that AI use is popular among anaerobic athletes, very little research has examined the effect of AI inhalation on muscular strength and power, with the results from those studies being contradictory (Table 1).

For example, one of the earliest peer-reviewed studies investigated the effect of AI inhalation on anaerobic muscular endurance on 25 college-aged males (Richmond et al., 2014). In this study, subjects inhaled AIs (or a placebo) for 3 seconds and then performed as many repetitions as possible (AMRAP) of the back squat (BS) with 85% of their 1-repetition maximum (1RM). After 5 minutes of rest, the same process was repeated but with the bench press (BP) exercise. There were no differences between the number of repetitions for either exercise after AI inhalation (compared to the same exercise with placebo), indicating that no ergogenic effect was found when performing an AMRAP protocol. However, although the authors only reported the number of repetitions performed, it is possible that the repetitions performed earlier in the AMRAP protocol may have been performed with greater velocity or power output, which would be an interesting follow-up study.

Another study (Perry et al., 2016) examined the effect of AI inhalation on muscle force, rate of force development (RFD), peak of force development (pRFD), and surface electromyography (EMG) during a maximal-effort isometric mid-thigh pull (IMTP) in 15 active males with no previous AI inhalation experience. The IMTPs were performed for 2 seconds immediately, 15, 30, and 60 seconds after AI inhalation (inhaled through the nostrils until an automatic withdrawal reflex has occurred) or no inhalation, followed by 5 minutes rest and performing the other condition in a counter-balanced order. Despite cerebrovascular and cardiovascular increases in the first 15 seconds after AI inhalation, there were no improvements in maximal force, pRFD, or EMG activity at any time. Therefore, it may be suggested there are no ergogenic effects of AI inhalation on maximal force production. However, a positive effect may have been shown on the pRFD, but post-activation potentiation (irrespective of AI use) cannot be ruled out in this case.

Similarly, another study investigated the influence of AI inhalation on the maximal force and pRFD during IMTP in addition to bodyweight countermovement jump power (CMJP) in 20 experienced resistance-trained men familiar with both weightlifting and powerlifting (Bartolomei et al., 2018). Subjects participated in three visits, separated 48 hours apart. During each visit, subjects performed 3 maximal effort bodyweight countermovement jumps (CMJ) with 3 minutes of inter-jump rest. Ten seconds before each jump, subjects inhaled AIs for 3 seconds (10 cm away from nostrils), a placebo, or no inhalation. Next, 3 maximal IMTP (6 seconds each) with 3 minutes rest between attempts were performed with a similar inhalation procedure. There was a significant increase in pRFD expressed during IMTP. However, no significant effects were found in CMJP or IMTP maximal force. Although the results did not indicate any improvement in maximal force production or vertical jump height, the potential ergogenic effect of AIs inhalation was shown by increasing explosive force output (e.g., pRFD).

One last peer-reviewed study investigated the effect of AI inhalation on the deadlift 1RM in 10 males and 10 females (Vigil et al., 2018). Subjects completed three visits separated by 72 hours rest. In the first visit, baseline deadlift 1RM and treatment (placebo and AIs) randomization occurred. The next two counter-balanced visits also included a traditional progressive 1RM protocol, but when attempting to lift 102.5%, 105%, and 107.5% of their baseline 1RM, subjects inhaled either AIs or placebo 15 seconds prior to the lift, as they normally would do in a powerlifting competition. Ultimately, there were no effects of AIs on deadlift 1RM in either men or women.

Although discussing conference abstracts is often excluded from review papers such as this one, the number of conference abstracts (6) far outweighs the number of peer-reviewed articles (4). Therefore, several conference abstracts are discussed in the following section.

For instance, the impact of AI inhalation on maximal effort CMJ height and broad jump (BJ) distance was investigated in 12 subjects (Mitchell et al., 2015). Before each jump, subjects randomly inhaled AI, a placebo, or nothing, but there were no differences in BJ and CMJ performance among conditions.

Similarly, another study investigated the effect of AIs on CMJ height and sprint time in 8 men and 3 women with at least 2 years of resistance training experience (Archer et al., 2017). Subjects performed 3 CMJs and 2 20m indoor sprints. Thirty seconds before each CMJ or sprint trial, subjects were instructed to take a deep breath through the nose of either an AI capsule, an AI liquid, or menthol oil. Similar to the previous study (Mitchell et al., 2015), there were no significant differences between conditions for any variable.

Another study investigated the effects of AI inhalation on RFD and peak force during IMTP in 8 males and 3 females with at least 2 years of resistance training experience (Rivera et al., 2017). Subjects attended 4 visits and performed IMTP after inhaling (30 seconds before lift) menthol oil, an AI capsule, an AI liquid, or nothing, in counter-balanced order before the performance. No significant differences were found for any variable.

The following 2 conference abstracts and 1 master's thesis were focused on determining the influence of AIs on anaerobic power. The first study (Secrest et al., 2015) investigated anaerobic mean power (MP) and peak power (PP) production using the 30 seconds Wingate test (with the resistance of 10% of each respective subject's body weight) before and after a simulated American football game (SAFG) on 10 healthy college-aged anaerobically trained males. Subjects attended two visits (AIs and control) separated by a minimum of 48 hours. Each SAFG was performed in randomized order and consisted of a total of 12, 9, or 6 maximal effort sprints, which lasted 5 seconds, and each sprint was followed by 40 seconds' break. Through each visit, subjects performed the Wingate test pre- and post-SAFG in a climate-controlled setting. During AIs inhalation visits, AIs were inhaled immediately before the post-SAFG Wingate test. There were significant differences in pre-SAFG MP and PP, and post-SAFG MP and PP after AIs inhalation. Although interesting, these findings have not been published in a peer-reviewed article and should be interpreted with caution.

On the contrary, the next study (Witherbee et al., 2013) investigated MP and PP during the 30 seconds Wingate test (with the resistance of 7.5% of each respective subject's body weight) on only 3 male participants with previous resistance training experience. Directly before the Wingate test, subjects inhaled a randomly chosen substance for 2 seconds (AIs, a placebo, or no inhalation). A minimum of 48 hours break was provided to separate each visit. There were not any significant differences.

Moreover, the following study (Groop, 2019) investigated MP, PP, and power drop (PD) by using the 30 seconds Wingate test (with the resistance of 11% of each respective subject's body weight) after AIs inhalation or no inhalation on 16 male ice-hockey players. Additionally, they also tested cardiorespiratory responses (HR and BR) and reaction time. Subjects took part in two visits, with randomized order, where they were instructed to inhale AIs or no inhalation. AIs were cracked 30 cm from nostrils and moved closer to the nostrils until a withdrawal reflex occurred whereas, during no inhalation visit, subjects performed a deep inhalation through nostrils without any AIs. After inhalation, there was a 60-second break, followed by reaction time tasks for an additional 6 minutes immediately followed by the Wingate test. Although a statistically significant cardiorespiratory response (increased HR and BR) occurred after 10 seconds after AIs, no changes were shown in any other variables.

In accordance with the presented results from various performance tasks, it may be suggested there are no ergogenic effects of AI inhalation on maximal muscular strength (Bartolomei et al., 2018; Perry et al., 2016; Richmond et al., 2014; Vigil et al., 2018). Moreover, no effects were observed on muscular endurance performance (Richmond et al., 2014) or in various explosive strength performances utilizing either slow (Archer et al., 2017; Bartolomei et al., 2018; Mitchell et al., 2015; Rivera et al., 2017) or fast stretch-shortening cycles (Archer et al., 2017). However, on the contrary, in the IMTP, which is an explosive isometric strength task utilizing a slow stretch-shortening cycle as well, significant improvements were noted in pRFD (Bartolomei et al., 2018; Perry et al., 2016). Ultimately, we can hypothesize that AI inhalation may improve pRFD while generating an explosive strength in IMTP performance whilst not in transferring it to any real-world dynamic exercise such as sprint, BJ, VL, or CMJ performances. However, peer-reviewed research should be conducted before confidently accepting this hypothesis.

In conclusion, regarding previous research, the highest significant pRFD (Bartolomei et al., 2018; Perry et al., 2016) and cardiorespiratory changes (Groop, 2019; Perry et al., 2016) were observed from 7.5 to 15 seconds after AIs inhalation, which agrees with the 15 to 30 second time window of the cardiorespiratory responses described earlier in this paper. Therefore, it appears that the timing of inhalation before performance may be considered as the most important variable when assessing the effects of AI inhalation on enhancing performance. Since maximal strength and explosive strength largely rely on the ATP-PC energy pathway (Rivera-Brown & Frontera, 2012), whereas the 30 seconds Wingate test and muscular endurance performance more or less rely on the combination of ATP-PC and the glycolytic energy pathways (J. C. Smith & Hill, 1991), any possible ergogenic effects of AIs inhalation would be more relevant for quick explosive movements. Nevertheless, further research needs to be done, and even though AIs showed several incidences of isolated ergogenic effects on explosive strength development and anaerobic power variables, the amount of data to date is far from convincing.

Table 1. Studies investigating ammonium inhalants effects on different performance.

Author, year	**Type**	**Sample size**	**Conditions**	**Dosing of substances**	**Distance, time of inhalation**	**Time from inhalation to test**	**Performance protocol**	**Findings**
(Richmond et al., 2014)	PRA	n = 25	AI, VVR	Liquid (AI) and gel (VVR) from microcentrifuge tubes	x, x	3 sec	Muscular endurance: BS and BP on 85% of 1RM AMRAP	⇔ BS and BP number of reps ⇔ BS and BP estimated 1RM
(Perry et al., 2016)	PRA	n = 15	AI, NI	Crushed AI capsule or deep inhalation of air	x, until a voluntary withdrawal reflex was observed	Immediately, 15, 30 and 60 sec	Cardiorespiratory: MCAv, HR, MAP, $PetCO_2$ Strength: PF, pRFD and RFD during IMTP	⇑ MCAv, HR ⇔ MAP, $PetCO_2$ ⇔ pRFD in IMTP ⇔ RFD in IMTP ⇔ PF in IMTP
(Bartolomei et al., 2018)	PRA	n = 20	AI, VVR, NI	Crushed AI capsule and liquid (VVR) from microcentrifuge tubes	10 cm, 3 sec	10 sec	Power: maximal effort CMJ Strength: PF, pRFD and RFD during IMTP	⇔ CMJ height ⇑ pRFD in IMTP ⇔ RFD, PF in IMPT
(Vigil et al., 2018)	PRA	n = 20 (10m, 10f)	AI, water	Crushed AI capsule or water from bottle	x, x	15 sec	Maximal strength: DL 1RM	⇔ DL performance
(Witherbee et al., 2013)	CA	n = 3	AI, VVR, NI	x	x, 2 sec	Immediately	Power: PP, PP per kg, MP, MP per kg, PD, PD per kg in WT	⇔ PP, PP per kg, MP, MP per kg, PD, PD per kg

(Mitchell et al., 2015)	CA	n = 12	AI, VVR, NI	x	x, x	Immediately	Power: VL height and BJ distance	⇔ VL height, BJ distance
(Secrest et al., 2015)	CA	n = 10	AI, NI	x	x, x	Immediately	Power: PP and MP in WT	⇑ PP and MP
(Rivera et al., 2017)	CA	n = 11 (8m, 3f)	AI, M, HPA, NI	x	x, x	30 sec	Strength: RFD and PF during IMTP	⇔ RFD and PF in IMPT
(Archer et al., 2017)	CA	n = 11 (8m, 3f)	AI, M, HPA, NI	x	x, x	30 sec	Power: VJ height and sprint time	⇔ VJ height and sprint time
(McEvoy et al., 2019)	CA	n = 22 (11 m, 11f)	AI, P	Crushed AI capsule	x, x	x	Respiratory: FEV1.0, FVC and MVV	⇔ FEV1.0, FVC and MVV
(Groop, 2019)	MT	n = 16	AI, NI	Crushed AI capsule or deep inhalation of air	5 cm, 3 sec	Immediately from cardiorespiratory, 1 minute from cognition and 6 minutes from power	Cardiorespiratory: HR and BR Cognition: reaction time Power: PP, MP, PD in WT	⇑ HR, BR ⇔ PP, MP, PD ⇔ reaction times

⇑ significantly greater ($p < 0.05$) than both placebo and no inhalation; ⇔ no significant difference ($p > 0.05$) compared with both placebo and no inhalation. *PRA = peer-reviewed article; CA = conference abstract; MT = master's thesis; m = men; f = female; AI = ammonia inhalant; P = unspecified placebo; VVR = Vicks VapoRub; M = menthol; NI = no inhalation; HPA = high potency ammonia; x = unspecified; PP = peak power; MP = mean power; WT = Wingate test; SAFG = simulated American football game; CMJ = counter movement jump; IMTP = isometric mid-thigh pull; pRFD = peak rate of force development; MCAv = middle cerebral artery blood flow velocity; HR = heart rate; MAP = mean arterial pressure; $PetCO_2$ = partial pressure of end-tidal CO_2; PF = peak force; RFD = rate of force development; BS = back squat; BP = bench press; 1RM = one repetition maximum; AMRAP = as many repetitions as possible; DL = deadlift; BR = breathing rate; FEV1.0 = one-second forced expiratory volume; FVC = forced vital capacity; MVV = maximum voluntary ventilation.

4.1.8. Research considerations

In summary, the effects of AIs on the cardiorespiratory system, cognitive functions, and strength and power performance are inconsistent, and the current amount of available data is insufficient to make confident conclusions regarding the efficacy of AIs. Furthermore, the mechanisms behind any anecdotal potential performance improvements are notably uncertain. When aiming to determine the efficacy of AIs in research settings, placebo and control conditions are often presented. Accordingly, the control condition often includes a deep inhale through the nostrils without any form of AI present. However, the placebo condition (the most frequently reported were menthol oil and Vicks VapoRub (VVR) which is used as a cough suppressant (Vicks Smart Label, 2020) and contains a compound of camphor (4.8%), eucalyptus oil (1.2%) and menthol (2.6%); (Table 1) has a distinct smell that subjects can likely distinguish from an AI. Although using such a substance for a placebo condition may seem scientifically sound, the presence of a placebo condition can also be viewed as a limitation, as subjects likely know that they did not inhale an AI. Simultaneously, it is crucial to note that while some ergogenic effects of AIs were observed, in no circumstances did the placebo condition or control condition result in an increase in performance superior to AIs inhalation. Therefore, there does not seem to be a placebo effect when considering AI inhalation.

4.2. Conclusion

In conclusion, AIs do not convincingly improve maximal muscular strength, muscular endurance, or reaction time performance. However, there is limited evidence that AIs can improve explosive strength pRFD during IMTP. Nevertheless, the AIs inhalation did no enhance performance in real-world dynamic movements like sprint time or CMJ height.

Moreover, AIs may have an ergogenic effect on anaerobic power performance, but only when the athletes were already fatigued. Despite this fact, it has not been investigated in different types of anaerobic power performance yet. Additionally, AIs likely produce significant cerebrovascular, cardiovascular, and respiratory responses 7.5 to 15 seconds after inhalation (possibly up to under 30 seconds), but then these variables decreased back to baseline values after 30 seconds. Therefore, it appears that the timing of inhalation before performance may be considered as one of the most significant conditions when using AIs as an ergogenic aid.

Furthermore, it is necessary to point out that a certain degree of relative risk may appear due to the possible occurrence of contact head injuries such as concussions. If an athlete inhales AIs right after experienced unconscious or dizziness after head contact, it may mask the symptoms, and the diagnosis of concussion would be complicated, but this notion is purely hypothetical and needs to be investigated. If that notion holds true, using AIs could lead to maintaining participation in the competition and worsen the consequences of head injury. Therefore, strength and conditioning and medical professional professionals should be cautious when athletes use AIs in these situations. On the other hand, the anecdotal evidence of AI inhalation indicates that athletes experience an optimal psychological state before a performance, and there is no evidence that AIs would decrease performance.

Ultimately, the athletes and the support staff around them should be knowledgeable about both the potential risks and beneficial effects of AIs inhalation during training and competition. To conclude, it appears crucial that more research could provide valuable insight into the effect of AIs inhalation on human physiology. Frequently, acute AI inhalation is compared with the delayed effect of caffeine supplementation. Hence, comparative research of those two ergogenic aids may help widen our understanding of the effects of these ergogenic aids. Moreover, future research may further focus on the determination of the level of psychological arousal by the electrodermal conductance response influenced by AIs inhalation. Also, considering a lack of research, more alternatives can be considered in terms of cognitive performance, and AIs inhalation, which would provide further essential findings in this particular topic.

4.3. Practical applications

- To date, the evidence does not support the use of ammonia inhalants to improve maximal muscular strength, muscular endurance, or reaction time. However, it may enhance 1) anaerobic power performance in already fatigued athletes, and 2) laboratory-tested explosive strength, but without any direct transfer to real-world dynamic exercise.

- The timing of ammonia inhalant use seems to be important, as heart rate and respiratory rate can increase up to 30 seconds after inhalation.

- Despite a plethora of anecdotal evidence, there is no research to directly support any increased psychological arousal in response to ammonium inhalants.

- Sport and health professionals should be aware of the potential risks and beneficial effects of ammonia inhalants during training and competition to prevent any unlikely, but possible, difficulties.

Using ammonia inhalants is widely prevalent among strength-based athletes. However, there is a major gap in the literature regarding its safety, physiological effects, and impact on physical performance.

5. The rationale for the second study

Following our review of the current literature regarding the benefits, risks, and efficiency of ammonia inhalants (Malecek & Tufano, 2021), the necessity to specify the most appropriate tests for evaluating the effects of using ammonium inhalants on military-specific physical and psychological performance became evident. Accordingly, shooting accuracy is one of our investigation's primary areas of interest. This skill is essential to every soldier, particularly for infantry forces, who often engage in frontline combat, where accurate shooting can be the difference between success and failure or even life and death (Mandache & Grigoras, 2022). Hence, the ability to maintain shooting accuracy, despite the adverse effects of sleep deprivation is a critical area of exploration and concern in our research context.

However, previous research endeavors into shooting performance have employed a range of heterogenous methodologies, with some studies utilizing rifle systems or live-fire handguns (B. Cohen et al., 2022; Enders et al., 2020; C. D. Smith et al., 2019; Ojanen et al., 2018; Barringer et al., 2018; Monaghan et al., 2017; S. A. T. Brown & Mitchell, 2017; T. N. Brown et al., 2016; Frykman et al., 2012; Swain et al., 2011; Tharion et al., 1997, 2003). Although these varied approaches provided a multitude of valuable insights, yet most provide no information about the shooting reliability and the familiarization of shooters with the shooting protocol. Therefore, there remained a gap in our understanding, emphasizing the need for methodological research specifically investigating the test-retest reliability of shooting protocols. Whereas by verifying this reliability, we wanted to ensure that our subsequent studies yield consistent and trustworthy results.

As all testing in the subsequent mains study (Chapter 8) was conducted in a controlled laboratory environment, we designed a shooting protocol using a laser-based shooting simulator. This required the development of a reliable test designed to meet our specific research goals. Therefore, our subsequent study titled *"Test-Retest Reliability of Two Different Laser-based Protocols to Assess Handgun Shooting Accuracy in Military Personnel"* (Malecek et al., 2023) was conducted.

Hence, the study aimed to assess the reliability of standard military shooting protocols using a laser-based handgun simulator. This involved twenty soldiers undertaking static and dynamic shooting trials on three different days to determine their shooting accuracy between-day and within-day consistency. The accuracy, denoted by total points, was recorded and analyzed for each protocol's reliability.

The primary rationale behind this study was to establish reliable protocols for assessing shooting accuracy under laboratory conditions that closely mimic the real-world demands faced by military personnel. The test-retest reliability design of the study ensures the consistency of the measurements over time, a critical component when evaluating the validity of any performance test. Furthermore, these protocols may serve as the foundation for future research examining the potential effects of other interventions on shooting performance. To a certain extent, choosing a laser-based shooting protocol allows us to capture accurate data in a safe, controlled, and repeatable environment. The selected protocols also allow for a realistic representation of the shooting tasks, which are crucial in the context of military performance.

By establishing these protocols, we also aim to set a basis for future studies investigating the effects of various interventions on shooting performance. Ultimately, our extended aim was to contribute valuable insights to the field of military performance optimization, potentially influencing shooting training practices in this high-stakes profession.

The following manuscript was initially submitted in 2022 to the BMJ Military Health Journal as an original research article. However, after undergoing the editorial review process, it was offered to submit a shortened version in the form of a letter to the editor, which was accepted in 2023. Therefore, to ensure consistency and uniformity throughout the entire dissertation thesis document, modifications have been made to the original manuscript's formatting. The substantive content of the original research article remains unchanged, and the final version of the letter to the editor is included as well. Adjustments have been made exclusively to aspects such as the citation style, table layout, figures, and graphs to align with this dissertation thesis's format specifications. Furthermore, the actual references have been restructured again and can be found enumerated at the culmination of this dissertation thesis document.

6. Test-Retest Reliability of Two Different Laser-based Protocols to Assess Handgun Shooting Accuracy in Military Personnel

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Institutional Review Board (Human Subjects): The Charles University Ethics committee approved the study (224/2020) (Appendix 7-8).

Data Availability Statement: Associated dataset, R script and JASP outputs for all performed analyses are available at the Open Science Framework [OSF] repository (https://osf.io/3n5w2/; OSF doi: 10.17605/OSF.IO/3N5W2).

List of abbreviations

Abbreviation	**Definition**
ICC	Intra-class correlation coefficient
CV	Coefficient of variation
SEM	Standard error of measurement
RM ANOVA	Repeated measures analysis of variance
SD	Standard deviation
OSF	Open science framework
CI	Confidence interval
MANUF	Modified version of the maneuver under fire

Letter to Editor

Tactical operations and armed conflicts involve symmetrical and asymmetrical conflicts, often occurring in urban and constantly changing environments. This requires tactical personnel to be prepared for unpredictable threats in dynamic scenarios (Clemente-Suárez & Robles-Pérez, 2013). In close-quarter situations, short weapons such as handguns are becoming increasingly prevalent over assault rifles, as they are more manageable for manoeuvring and battling. Therefore, accurate handgun shooting is essential for tactical personnel who may encounter close-quarter situations requiring precise shooting. Although regular handgun training is necessary to improve static and dynamic shooting accuracy, implementing basic shooting training is challenging as it includes budget constraints, ammunition costs, and limited access to live-fire ranges, necessitating the development and use of new technologies to meet current shooting training standards.

Laser-based shooting simulators have emerged as an alternative to traditional live-fire shooting, offering several benefits such as reduced ammunition and target costs, safety, decreased waste, and more frequent and time-efficient training exercises (e.g., no need to replace targets, assemble and clean guns, transports to shooting ranges) (Hagman, 1998). Previous researchers have used laser-based systems to evaluate shooting performance under different conditions, such as supplementation, sleep deprivation, and psychological or physiological fatigue. However, most studies lacked information on reliability and shooter familiarization with the protocol. Consequently, the results of those investigations should be interpreted cautiously as the shooting protocols themselves may not have been reliable, possibly affecting the observed effects. Therefore, this study aimed to assess the reliability of two standard-issued shooting protocols using a laser-based handgun system.

Twenty soldiers (22.0 ± 1.9 yrs) participated in two static and dynamic shooting protocols trials on three separate days to determine the between-day and same-day test-retest shooting accuracy reliability (Figure 1). The accuracy (total points scored) for each trial was recorded and analysed for 1) the reliability of each shooting protocol with two-way mixed effects intra-class correlation coefficient (ICC) with a coefficient of variation (CV) and the standard error of measurement (SEM) and 2) differences in shooting accuracy points scored between days and trials by two-way RM ANOVA. Associated literature review, ethical approval, methods, analyses, and supplements are available at the Open Science Framework (https://osf.io/3n5w2).

The results indicated good between-day test-retest reliability of the average of two trials of both the static (ICC = 0.837 [0.659, 0.930], CV = 3.78%, SEM = 3.37) and dynamic (ICC = 0.806 [0.597, 0.917], CV = 4.73%, SEM = 3.73) protocols. Additionally, there was moderate between-day test-retest reliability of a single trial for static (ICC = 0.703 [0.383, 0.872], CV = 3.47%, SEM = 3.11) and dynamic (ICC = 0.585 [0.219, 0.810], CV = 4.17%, SEM = 3.30) protocols, and moderate same-day test-retest reliability for static (ICC = 0.510 [0.248, 0.741], CV = 2.57%, SEM = 2.31) and dynamic (ICC = 0.510 [0.243, 0.742], CV = 4.30%, SEM = 3.39) protocols across the last two trials (Figure 4).

Our study demonstrates that soldiers' shooting performance, based on accuracy in static and dynamic protocols, has moderate to good reliability and no statistically significant effect on the difference in shooting accuracy between days and trials. Therefore, the protocols used in this study and the reliability observed may serve as a foundation for future research to establish a more rigorous approach using validated shooting protocols. By following these guidelines, researchers may contribute to the advancement of shooting-related research, ultimately leading to more accurate shooting assessments and training.

Original research

Abstract:

Shooting is a fundamental skill for any soldier and is crucial in military combat operations. In contemporary warfare, the use of handguns over assault rifles in close combat scenarios is becoming increasingly prevalent. As a result, evidence-based training and research on factors influencing handgun shooting performance are paramount. However, data on the reliability of standard static and dynamic protocols are lacking. The present study aims to evaluate the test-retest reliability of standard-issued static and dynamic military shooting protocols executed using a laser-based handgun simulator system. Twenty soldiers (22 ± 1.9 yrs; 81.7 ± 8.6 kg; 184.9 ± 5.9 cm) participated in two trials of both static and dynamic shooting protocols on three separate days in order to determine the between-day and within-day test-retest reliability of their shooting accuracy. The accuracy (total points scored) for each trial was recorded and analyzed for the reliability of each shooting protocol with two-way mixed effects intra-class correlation (ICC) for consistency. Our results indicate good between-day test-retest reliability of both the static (ICC = 0.837 [0.659, 0.930]) and dynamic (ICC = 0.806 [0.597, 0.917] protocols. Additionally, there was a moderate between-day (for static ICC = 0.703 [0.383, 0.872] and dynamic ICC = 0.585 [0.219, 0.810] protocols) and same-day (for static ICC = 0.510 [0.248, 0.741] and dynamic ICC = 0.510 [0.243, 0.742]) reliability across two trials. In conclusion, our study demonstrates that soldiers' shooting performance, based on accuracy in static and dynamic shooting protocols, has moderate to good reliability. Therefore, the present study and its protocols may serve as a foundation for future researchers and practitioners to establish a scientific approach for validating the reliability of shooting protocols. By following these guidelines, researchers and practitioners may contribute to the advancement of shooting-related research, ultimately leading to more accurate and reliable shooting assessments and training programs.

Keywords: Army; Soldiers; Weapon; Simulator; Firearm

Key messages:

- Despite many studies using laser-based systems to assess shooting performance under various conditions, few studies have explicitly reported the reliability of shooting protocols, indicating a need for more reproducible shooting protocols.
- The present study provides evidence of moderate to good reliability in soldiers' shooting performance, as assessed by accuracy in static and dynamic shooting protocols, indicating that the study protocols can serve as a basis for establishing a scientific approach to validate the reliability of shooting protocols for future research and practical applications.

- Despite many studies using laser-based systems to assess shooting performance under various conditions, few studies have explicitly reported the reliability of shooting protocols, indicating a need for more reproducible shooting protocols.

6.1. Introduction

Tactical operations and armed conflicts can be characterized as a combination of traditional symmetrical (with fairly defined targets and tactics) or less traditional and asymmetrical conflicts. Asymmetrical conflicts often occur in urban areas with a constantly changing environment (Clemente-Suárez & Robles-Pérez, 2013). Thus, tactical personnel must be prepared for unpredictable threats and attacks in a dynamic scenario (Clemente-Suárez & Robles-Pérez, 2013). For solving such armed conflicts, short weapons are likely preferred over assault rifles as they are more manageable in maneuvering and battling in close quarters where the shooting distances are likely less than 7 meters (Clemente-Suarez & Robles-Pérez, 2015; Nieuwenhuys & Oudejans, 2010). Considering the above, handguns are becoming the most likely weapon of choice for soldiers, emergency tactical personnel, patrol soldiers, or other enforcement officers who may encounter close-quarter situations where both static and dynamic handgun shooting (likely including multiple shots to eliminate targets) must be precise and accurate.

Handgun training should be regularly performed to improve the accuracy of both static and dynamic shooting. However, regularly implementing a basic shooting training system is challenging for various reasons (General Accounting Office Report to Congressional Committees, 1995). Standard issue rifles or handguns with live ammunition and disposable targets are typically used during live-fire shooting exercises. Budget restrictions, limited availability of live-fire ranges and increasing expenditures on ammunition have all but necessitated the development and widespread use of new technologies to fulfill current shooting training standards (Krug & Pickrell, 1996).

Laser-based shooting training simulators are used by military personnel, police officers, and shooting athletes as a potential replacement for a traditional live-fire shooting range (Hagman, 1998). Not only can laser-based handgun systems reduce ammunition and target costs, improve safety, and decrease the amount of waste generated by live-fire exercises, but they may also significantly enhance the quality of shooting training by allowing for more frequent and time-efficient exercises (i.e., no need to replace targets, assemble and clean the gun, transports to live-fire shooting range, etc.). Lastly, laser-based systems allow for the performance of all shot process functions, such as time and distance to the target or landing of individual hits, to be easily and precisely recorded and analyzed. This could lead to the future development of correct targeting techniques and practical behavior skills under realistic tactical scenarios.

Many studies have used laser-based systems to assess shooting performance while investigating the effects of various conditions such as supplementation (Barringer et al., 2018; Monaghan et al., 2017; Tharion et al., 1997, 2003), lack of sleep (B. Cohen et al., 2022; C. D. Smith et al., 2019), neurocognitive training (Enders et al., 2020), psychological and physiological fatigue (Frykman et al., 2012; Ojanen et al., 2018; Swain et al., 2011) or different military clothing and equipment (S. A. T. Brown & Mitchell, 2017; T. N. Brown et al., 2016). Many of these studies included multiple trials and averages the scores together without first establishing the reliability (Monaghan et al., 2017). Throughout the publicly available literature (and to the authors' best knowledge), only one study (Kelley et al., 2020) has explicitly stated the reliability of their laser-based shooting tests. Specifically, this study shows that among four dynamic rifle shooting tasks, the reliability of shooting accuracy ranged from ICC = 0.44 to 0.78 (Kelley et al., 2020). Vast majority of aforementioned studies provide no information about the shooting reliability and non on the familiarization of shooters with the shooting protocol. As a result, we must interpret the results of those investigations with some caution, as it may be possible that the shooting protocols themselves may not be reliable possibly affecting the reliability of observed effects.

Therefore, there seems to be a clear need for reproducible shooting protocols in order to determine the effects of various conditions on shooting performance with more confidence. As mentioned above, only one study (Kelley et al., 2020) reported the reliability of four dynamic laser-based shooting tests. But they were using a rifle, greater engagement distances, and did not include static shooting (Kelley et al., 2020). Even though one of those dynamic protocols reported good reliability, modern tactical conflicts occur in close-quarters, thereby increasing the need to establish reliability of handgun shooting protocols that can be also performed with laser-based systems. Therefore, this study aimed to explore the test-retest reliability of basic static and dynamic shooting protocols carried out with a laser-based handgun simulator system. Considering that the measurements were carried out in laboratory-based conditions and under standardized manners highly limiting any potentially confounding variables, we hypothesized that both protocols would be reliable. This can ultimately guide researchers and practitioners in choosing reliable testing procedures that can be used during shooting testing or training.

6.2. Methods

6.2.1. Participants

Twenty active-duty male cadets (22 ± 1.9 yrs; 81.7 ± 8.6 kg; 184.9 ± 5.9 cm; all reported as mean ± standard deviation) serving at the University military department participated in this study. Eligibility criteria for participation included having passed an annual physical fitness test and medical check-up within the previous year, having more than two years of active-duty service experience, self-reported high levels of comfort with shooting a handgun (having completed basic military handgun shooting training but not being explicitly trained as marksmen), and not currently working shiftwork or taking medications known to affect cognitive or physical performance.

6.2.2. Study design

A repeated-measures design was employed to evaluate the reliability of handgun shooting accuracy during both static and dynamic shooting protocols, with a focus on both between-day and same-day test-retest reliability. Participants were required to visit the laboratory on five separate occasions. During the initial visit, participants were provided with information regarding the benefits and risks of the investigation and were required to sign an informed consent (the study was approved by the Faculty ethics committee; approval number 224/2020). The second visit was familiarization with the testing procedures. In the subsequent three experimental sessions, participants completed two trials of both the static and dynamic shooting protocols on three distinct days over the course of a week (Figure 1).

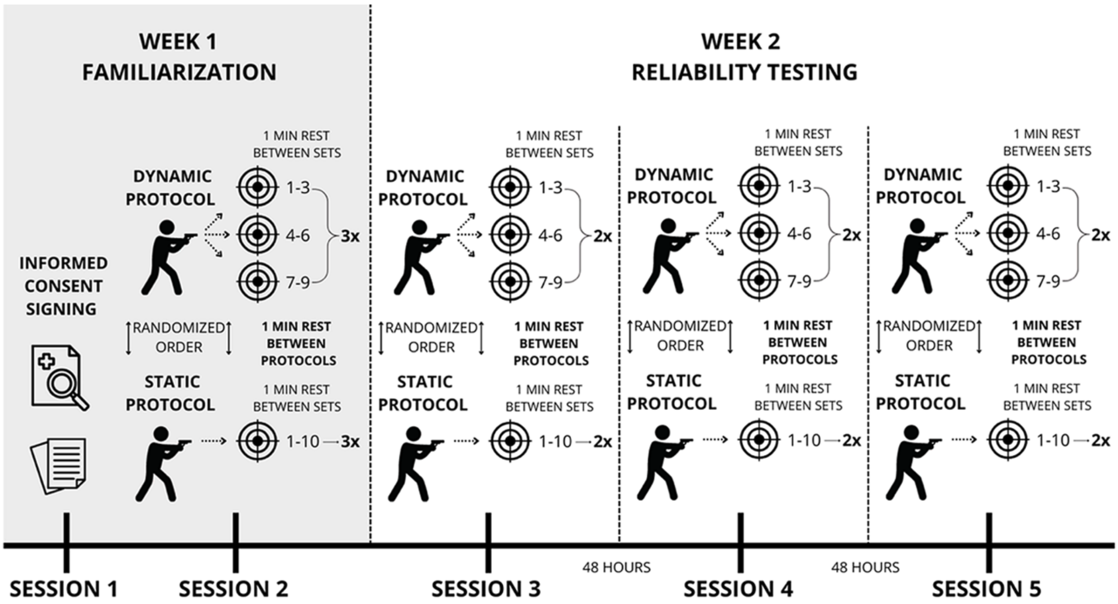

Figure 1. Timeline of the data collection.

6.2.3. Familiarization

During the second visit, the research team familiarized participants with all procedures and details related to both shooting protocols. Team members (JM, DO, ZD) demonstrated and explained the standardized isosceles high-ready stance position (i.e., feet parallel at shoulder-width with both arms extended, holding the handgun out in front at eye level ready to engage; Figure 2) (Lewinski et al., 2015). Participants were also instructed on the proper handling of the laser system handgun while shooting, practiced shooting while wearing headphones, and were informed of the instructions they would receive during the shooting protocols. Once the participants felt comfortable and confident, they completed three trials of each shooting protocol in a randomized order. In addition, they performed one familiarization trial of each shooting protocol before each experimental session in a randomized order.

Figure 2. Example of isosceles stance positioning during the handgun shooting protocol.

6.2.4. Experimental testing

Each of the three testing sessions was conducted at the same time of day, with at least 48 hours separating each session. Each session consisted of two trials of the static shooting protocol and two trials of the dynamic shooting protocol. All trials were performed in a standardized isosceles stance position, as illustrated in Fig 1. A laser-based simulator system (LASRX, Plano, TX & Beatrice, NE) equipped with an infrared laser handgun (SIRT 110, Next Level Training, Ferndale, WA) was used in this study. For this study, the handgun used was a real-weight mock-up of a Glock 17/22 with iron sights, which is a standard-issue handgun for the Czech military. During testing, participants wore over-ear headphones to hear the software command to start shooting and the simulated handgun shooting blasts when pulling the trigger.

For both shooting protocols, a 20 cm circular target was placed on a blank wall 4 meters in front of participants to simulate a 50 cm target 10 meters away (Swain et al., 2011). Each experimental session consisted of 2 trials of each (static and dynamic) protocol. First, participants performed one practice trial of each protocol in a randomized order with 1-minute rest between trials. Next, they either performed two consecutive trials of the static protocol or two consecutive trials of the dynamic protocol for data collection purposes. Each trial and each protocol were all separated by one minute (the order of static and dynamic protocols were randomized among participants). For the static protocol, they fired 10 consecutive shots, aiming to hit the middle of the circular target (a bullseye was worth 10 points, and 1 point was deducted for every 2 cm region away from the bullseye, resulting in a theoretical maximum score of 100 points). For the dynamic protocol, participants fired 3 shots in a row at 3 targets from left to the right for a total of 9 shots (i.e., simplified "El Presidente" protocol) (Lane, 2019; McNamara et al., 2016). The middle target was positioned identically as the one in the static protocol, with the right and left targets positioned at a bullseye-to-bullseye distance of 1-meter from the center target in the same horizontal plane. The scoring system was the same as for the static protocol, resulting in a maximum score of 90 points. Participants were instructed to try to shoot as accurately as possible within a maximum time limit of 1-minute per trial. The sum of points from each trial for each participant of each shooting protocol was recorded for subsequent reliability analyses (Table 2).

Table 2. Descriptive statistics of the sample shooting performance in static and dynamic protocol across days (1-3), and trials (a, b) (SD = standard deviation). Scores are on a scale of 0-100 in static and 0-90 in dynamic shooting protocol.

Task	Day	Trial	Mean ± SD	Skewness	Minimum	Maximum
Static shooting protocol	1	a	87.85 ± 5.97	- 1.01	74	94
		b	88.60 ± 5.39	- 1.59	75	95
	2	a	89.20 ± 5.04	- 0.10	81	97
		b	90.15 ± 4.21	- 0.42	80	97
	3	a	90.15 ± 4.84	- 1.45	76	96
		b	90.00 ± 3.66	- 0.80	81	95
Dynamic shooting protocol	1	a	77.40 ± 5.50	- 0.58	65	84
		b	78.65 ± 5.26	- 2.20	61	84
	2	a	79.30 ± 4.85	- 1.40	67	84
		b	79.75 ± 4.17	0.04	72	87
	3	a	78.25 ± 5.89	- 0.94	63	86
		b	79.70 ± 4.73	- 0.56	71	86

6.3. Statistical analysis

6.3.1. Reliability analyses

The ICCs were calculated using the irr package (version 0.84.1) (Gamer et al., 2019) in R (version 4.2.0) and were reported together with their 95% CI [LL, UL]. We interpreted the ICCs values as poor (values less than 0.50), moderate (between 0.50 and 0.75), good (between 0.75 and 0.90), and excellent (greater than 0.90) (Koo & Li, 2016). We performed three different ICC analyses separately for both the static and dynamic protocols, thus six in total.

First, to assess the between-day test-retest reliability of the average accuracy score (between-day / average), we calculated the ICC for three average accuracy scores from three individual testing days, where each score included the average of two shooting trials at that given time point. For this, we used the ICC (A,k) formula (two-way mixed effects, absolute agreement, average measurement) (McGraw & Wong, 1996).

Second, to assess the between-day test-retest reliability of a single trial (between-day / single), we calculated the ICC for three accuracy score from three individual days, where each score was the result of the second shooting trial at that time point (e.g., treating the first trials as additional familiarization). For this, we used the ICC (A,1) formula (two-way mixed effects, absolute agreement, single measurement) (McGraw & Wong, 1996).

Third, to assess the same-day test-retest reliability across two trials (same-day / average), we calculated the ICC for two accuracy scores from the same day, where each score was the result of the two shooting trials from the final day (e.g., treating the first two days as additional familiarization), using the ICC (A,1) formula (two-way mixed effects, absolute agreement, single measurement) (McGraw & Wong, 1996).

6.3.2. Differences in mean shooting performance

The analysis of the difference in shooting accuracy points scored between days and trials were conducted using JASP (version 0.16.2, 2022). Parametric tests were performed once the normality assumptions were verified using the Shapiro-Wilk W test. Data were analyzed using a one-way repeated measures ANOVAs separately for both static and dynamic shooting protocols (between-day test-retest reliability of the average accuracy score, 4 time: [Day 1a, Day 1b, Day 2a, Day 2b, Day 3a, Day 3b]; between-day test-retest reliability of a single trial, 3 time: [Day 1b, Day 2b, Day 3b]; the same-day test-retest reliability across two trials [Day 3a, Day 3b]). The assumption of sphericity was assessed using Mauchly's W. In cases where sphericity assumptions were violated, a Greenhouse–Geisser adjustment was applied. The variance explained by each RM ANOVA model is reported in η^2. When the ANOVA tests demonstrated a statistically significant main effect of time, post-hoc comparisons (with Bonferroni correction) of the mean differences were performed.

6.3.3. Sample size justification

We calculate the sample size required to observe an intra-class correlation coefficient (ICC) of 0.8 with lower bound of the 95% CI greater than 0.5 (the threshold for moderate reliability) based on precision power analysis. To do so we used the ICC.Sample.Size package (version 1.0) in R (version 4.2.0) (R Core Team, 2014; Zou, 2012). Assuming three testing per participant, a desired power of 80%, and using a two-sided 0.05 significance level, the required sample size would be 18. To account for possible drop-outs or technical issues, we recruited 20 participants.

6.3.4. Data availability statement

Associated dataset, R script and JASP outputs for all performed analyses are available at the Open Science Framework [OSF] repository (https://osf.io/3n5w2/; DOI 10.17605/OSF.IO/3N5W2).

6.4. Results

In the static protocol, the ICC (A,k) for between-day test-retest reliability of the average of two trials was 0.837 [0.659, 0.930], the ICC (A,1) for between-day test-retest reliability of a single trial was 0.703 [0.383, 0.872], and the ICC (A,1) for same-day test-retest reliability across two trials was 0.510 [0.248, 0.741] (Figure 3A).

For the dynamic protocol, the ICC (A,k) for between-day test-retest reliability of the average of two trials was 0.806 [0.597, 0.917], the ICC (A,1) for between-day test-retest reliability of a single trial was 0.585 [0.219, 0.810], and the ICC (A,1) for same-day test-retest reliability across two trials was 0.510 [0.243, 0.742] (Figure 3B). The comparison of all ICCs and their CIs can be observed in Figure 4.

There was no statistically significant main effect of time for the analysis of the difference in shooting accuracy points scored between days and trials (Table 3).

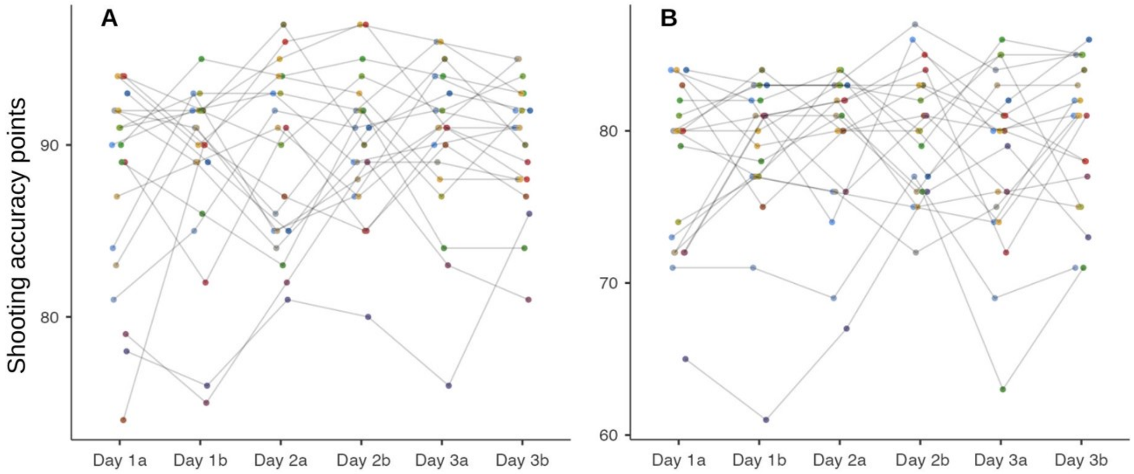

Figure 3. Individual shooters‘ score in the static **(A)** and dynamic **(B)** shooting protocol across days (1-3) and trials (a, b). Color dots depict each participant with grey lines connecting individual scores across days and trial. Scores are on a scale of 0-100 in static and 0-90 in dynamic shooting protocol.

Table 3. Within subject effects of individual test-retest reliability tests for static and dynamic shooting protocols.

Task	Mean differences comparison	F (df)	p	η^2
Static shooting protocol	between-day of the average accuracy score	1.588 (5)	0.171	0.077
	between-day of a single trial	1.509 (2)	0.234	0.074
	same-day across two trials	0.040 (1)	0.844	0.002
Dynamic shooting protocol	between-day of the average accuracy score	1.218 (5)	0.307	0.060
	between-day of a single trial	0.695 (2)	0.505	0.035
	same-day across two trials	1.820 (1)	0.193	0.087

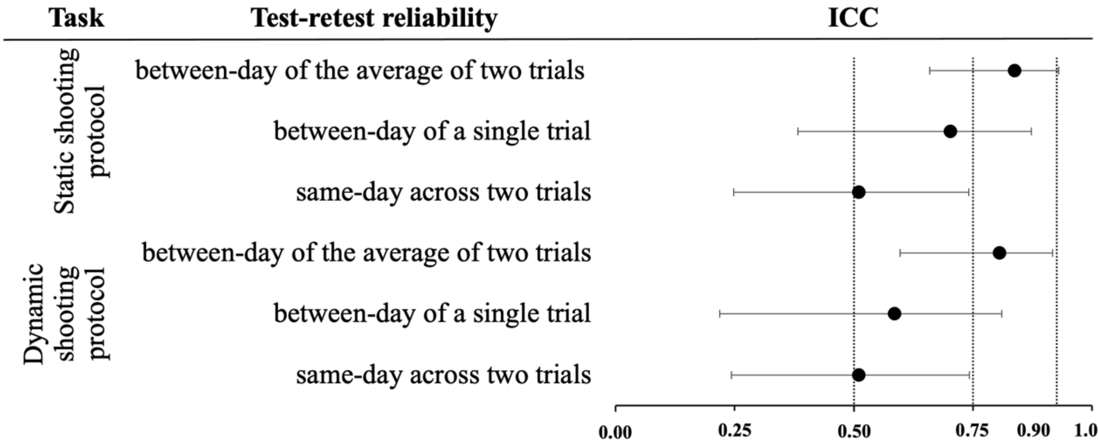

Figure 4. Intraclass correlation coefficients (ICC) of test-retest reliability for static and dynamic protocols. Note: Black dots represent mean ICC, error bars their 95% CI. The dotted bands represent areas of poor (< 0.50), moderate (0.50 - 0.75), good (0.75 - 0.90), and excellent (> 0.90) agreement.

6.5. Discussion

The current study aimed to assess the test-retest reliability of static and dynamic shooting protocols utilizing a laser-based handgun simulator system. The results of the test-retest reliability analyses indicate that our participants displayed good between-day reliability for both the static and dynamic shooting protocols when utilizing averaged scores. Additionally, moderate between-day (i.e., the second trial from each Day 1-3) and same-day (i.e., the two trials on Day 3) reliability was observed for both static and dynamic protocols. Furthermore, we observed no differences in shooting accuracy scores between or within days. Thus, future research could utilize these protocols as sufficiently reliable to examine shooting performance in military personnel and other tactical populations.

To the best of our knowledge, this is the first study to examine the test-retest reliability of handgun shooting protocols, thus, no direct comparisons can be made to previous studies. However, similar studies have been conducted in the past. For instance, one study (Kelley et al., 2020) evaluated the test-retest reliability of a newly-developed dynamic laser-based marksmanship range using a rifle. In their study, the test-retest reliability was assessed on two consecutive days, where soldiers (N = 40) were instructed to shoot during four different dynamic scenarios. The reliability observed varied from ICC = 0.44 to 0.78, which was generally somewhat lower compared to the reliability observed in the present study (ICCs range of 0.510 to 0.837 for static and 0.510 to 0.806

for dynamic protocols). At first glance, these finding of slightly lower consistency in rifle shooting may seem surprising. A rifle, due to its greater barrel length and enhanced support from shooter's shoulder and hands, should provide more stability and accurate shooting results, compared to the set-up used in the present study, where a handgun was held in outstretched arms. Despite that, the between-day test-retest reliability of the average accuracy scores of our tested dynamic handgun shooting protocol was slightly greater (ICC = 0.806) than the dynamic rifle shooting protocol of the previous study (ICC = 0.780) (Kelley et al., 2020). This can likely be attributed, in part, to the differences in shooting distance in both studies. In the present study, targets were positioned to simulate a distance of 10 meters (e.g. a typical engagement distance for a handgun "close-quarters battle" scenario) (Clemente-Suarez & Robles-Pérez, 2015; Nieuwenhuys & Oudejans, 2010), whereas previous study (Kelley et al., 2020) included targets ranging from 15 to 75 meters (targets size were not specified). Therefore, even if the rifle were theoretically more stable, small imprecisions in aiming magnify over distance and can result in comparably less accurate or less precise shooting at greater distances.

In addition to shooting distance, the other factor that may have contributed to the greater reliability observed in the present study may be the less dynamic character of our 'dynamic' shooting protocols compared to those used in the previous study using a laser-based rifle system (Kelley et al., 2020). In the present study, participants stood still and shot at different targets next to each other, resulting in only slight head, trunk, arm, and handgun movement in the horizontal plane. The dynamic protocols in the rifle laser-based study included more extensive movements, such as walking, kneeling, picking up a rifle, rotating around an axis, and traversing across a narrow beam (Kelley et al., 2020). With so many factors at play, combined with the greater shooting distances, it is not surprising that the reliability of our 'dynamic' protocol was greater than the previous study (Kelley et al., 2020). Additionally, our static shooting protocol resulted in similar test-retest reliability as our dynamic shooting protocol, possibly because the dynamic protocols included very little movement. However, it should also be noted that the protocol with the highest reliability in the previous study (Kelley et al., 2020) was the least dynamic one, which, unlike the other protocols that required walking or picking up the rifle from the floor, involved only taking a narrow kneeling stance and shooting in different directions. Therefore, future researchers and practitioners should be aware that additional independent variables, such as body position, movement, different distances, and extra tasks to accomplish while shooting, would likely affect the reliability of the test.

The highly standardized and thus less ecologically valid environment of the present study can be perceived as both a strength and a weakness, depending on the context. For instance, previous research has employed laser-based simulators to investigate the impact of various external factors (Barringer et al., 2018; S. A. T. Brown & Mitchell, 2017; T. N. Brown et al., 2016; B. Cohen et al., 2022; Frykman et al., 2012; Monaghan et al., 2017; Ojanen et al., 2018; C. D. Smith et al., 2019; Swain et al., 2011; Tharion et al., 1997, 2003) on shooting performance. In particular, one study (Jaworski et al., 2015) examined the effects of load carriage on the performance of combat-related tasks and shooting accuracy. The soldiers (N = 12) in this study underwent a modified version of the Maneuver Under Fire (MANUF) portion of the U.S. Marine Corps Combat Fitness Test, conducted in four different load conditions (no load, 15%, 30%, and 45% of body weight) with a static kneeling shooting accuracy test conducted both before and after each MANUF condition. Although the study results indicated that overall shooting accuracy was not affected by acute load (pre-MANUF; $p = 0.160$), overall statistically significant decrements were reported post-MANUF ($p = 0.005$). Therefore, the authors concluded that combat-relevant fatigue might be the underlying cause of decreased shooting accuracy performance in soldiers. However, it should be noted that this study did not provide information on the shooting protocol reliability data or a familiarization. As a result, it is necessary to interpret the outcomes of this study, and others like it, with caution, as it is possible that the shooting protocols themselves may not have been sufficiently reliable.

Thanks to the technological advancements and recognition that laser-based shooting simulators can serve as a substitute for live-fire shooting performance (Crowley et al., 2014; Hagman, 1998), many researchers now employ various forms of laser-based shooting simulators, the majority of which are rifles. It is worth noting that, owing to the vast array of available simulator systems, a multitude of additional queries may arise. One of the principal distinctions among simulators is the implementation of a recoil system. When there is no recoil system, soldiers do not experience and do not have to anticipate the forces of recoil and muzzle-rise, as they would during live fire. Most laser-based systems also lack other effects, such as noise and muzzle flash, which, if present, could cause some shooters to flinch in anticipation of a shot, thus making the shooting experience more comfortable and less ecologically valid than when using a recoil system or live-fire. On the other hand, with more advanced features, the cost of the simulator systems can increase, and portability may decrease, which can make sophisticated simulators similarly difficult to utilize for research as live-firing. Furthermore, the reduced portability (e.g., a need for wired tether) may also decrease the utility of simulators in more dynamic protocols that are closer to the real-world environment of modern warfare.

The use of a wireless handgun systems that emits an invisible laser may allow for creation of more dynamic data collection scenarios, such as the ability for a soldier to move between multiple firing locations or to quickly transition between firing positions within a given scenario. These dynamic components may enable future research to yield additional insight into the impact of various conditions on handgun shooting performance. Previous research has suggested that future studies should investigate the use of more portable and cost-effective laser-based systems in order to examine the effects of various conditions on shooting performance (Kelley et al., 2020). In line with that, the present study utilized a "lower-cost" LASRX system. Though LASRX uses a wireless invisible laser handgun, the system depends on a camera wire-connected to a computer to collect data, rendering its use for dynamic protocols limited.

In conclusion, it is necessary to note that the previous research has demonstrated a positive correlation (r = 0.69) between the performance of a laser-based shooting simulator and that of live-fire shooting, suggesting that it could serve as a valuable tool for predicting shooting aptitude (Hagman, 1998). However, the study also cautions against using laser-based simulators as a replacement for live-fire training, given the significant dissimilarities, such as the influence of natural gun recoil, between simulated and live-fire exercises, which can affect shooting skills in the long term. Nonetheless, it should be emphasized that the purpose of this study was not to compare laser-based shooting and live-fire shooting simulators or to draw attention to the absence of recoil or the accessibility and portability of specific systems.

Rather, this study aimed to highlight the importance of establishing the reliability of basic shooting protocols employed in research to assess the impact of various factors on shooting accuracy. Accordingly, a basic static shooting protocol (Ojanen et al., 2018), which is the standard method used by military organizations for training and testing shooting accuracy, along with a basic dynamic protocol designed for close-range combat practice, were selected. Thus, the results of present study and its basic protocols can serve as a foundation for future researchers and practitioners to develop and apply a systematic and scientific methodology for validating the reliability of their shooting protocols.

6.6. Conclusion

The present study indicates that the selected static and dynamic shooting protocols performed with an infrared laser handgun in a laser-based system exhibit moderate to good test-retest reliability. Nevertheless, validating the selected shooting protocols using live-fire handguns in future research is crucial. Additionally, it is recommended that other protocols and laser-based systems to be examined to address diverse shooting requirements during training and deployment.

7. The rationale for the third study

In recent years, there has been a growing scientific interest in the effects of sleep deprivation on performance, particularly in demanding fields such as the military (Petrofsky et al., 2021; Grandou et al., 2019; Grugle et al., 2004). The adverse effects of sleep deprivation on cognitive and physical performance, which have been well-documented in the scientific literature (Petrofsky et al., 2021; Grandou et al., 2019), present a significant concern in the context of military operations, given that military personnel frequently operate under challenging conditions with a limited amount of sleep.

In our first research (Chapter 4), we reviewed the current existing literature on the use of ammonia inhalants as a potential ergogenic aid. These inhalants, widely used among strength-based athletes (Herrick & Herrick, 1983; Prewitt, 2016; Pritchard et al., 2014; Rivera-Brown & Frontera, 2012), are believed to trigger the adrenergic receptors in peripheral tissues by irritating the respiratory passages (Velasquez, 2011). This action increases cardiac output, respiratory rate, and blood flow velocity in the middle cerebral artery (Perry et al., 2016). Consequently, ammonia inhalation has been associated with enhanced alertness, perceived performance, explosive strength during isometric muscle actions (Bartolomei et al., 2018), and repeated anaerobic power performance, particularly in already fatigued individuals (Secrest et al., 2015).

Given the substantial demands placed on military personnel and the pervasive issue of sleep deprivation in military contexts, we aimed to explore the potential positive effects of fast-acting ergogenic aid in the form of ammonia inhalants on sleep deprivation-induced performance decrements. The primary aim of our study, *"Effects of 36 hours of sleep deprivation on military-related tasks: can ammonium inhalants maintain performance?"*, was to examine whether the use of ammonia inhalants can offset the adverse cognitive and physical effects associated with 36 hours of sleep deprivation in the performance of military-related tasks. By providing evidence-based information on this topic, we aimed to contribute to a more nuanced understanding of the potential benefits and limitations of ammonia inhalants as ergogenic aids in sleep-deprived conditions. Furthermore, we hoped to provide valuable insights into potential optimization of performance under challenging conditions.

This manuscript is currently under review in the PLOS ONE as clinical trial research registered in ClinicalTrials.gov (NCT05868798). The substantive content of the manuscript remains unchanged. However, modifications have been made to the original manuscript's formatting to ensure consistency and uniformity throughout the entire dissertation thesis document. Minor changes have been made exclusively to aspects such as the citation style, table layout, figures, and graphs to align with this dissertation thesis's format specifications. Moreover, the reference list has undergone a reorganization and can now be located in a numbered format at the end of this dissertation thesis document.

8. Effects of 36 hours of sleep deprivation on military-related tasks: can ammonium inhalants maintain performance?

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Institutional Review Board (Human Participants): The National Institute of Mental Health ethics committee approved the study (approval number 176/20), and all participants signed written institution-approved informed consent (Appendix 9-10).

List of abbreviations

Abbreviation	**Definition**
AI	Ammonia inhalants
TSD	Total sleep deprivation
ESS	Epworth sleepiness scale
SRT	Simple reaction time
SA	Shooting accuracy
DAS	Rifle disassembling and reassembling
JH	Countermovement jump height
HR	Heart rate
RPE	Rating of perceived exertion
CON	Intervention without ammonia inhalants
PEBL	Psychology Experiment Building Language
bpm	Beats per minute
CMJ	Unloaded countermovement jump
ANOVA	Analysis of variance
SD	Standard deviation
OSF	Open science framework
CI	Confidence interval
LL	Lower limit
UL	Upper limit

Abstract

A lack of sleep can pose a risk during military operations due to the associated decreases in physical and cognitive performance. However, fast-acting ergogenic aids, such as ammonia inhalants (AI), may temporarily mitigate those adverse effects of total sleep deprivation (TSD). Therefore, the present study aimed to investigate the acute effect of AI on cognitive and physical performance throughout 36 hours of TSD in military personnel. Eighteen male cadets (24.1 ± 3.0 y; 79.3 ± 8.3 kg) performed 5 identical testing sessions during 36 hours of TSD (after 0 [0], 12 [-12], 24 [-24], and 36 [-36] hours of TSD), and after 8 [+8] hours of recovery sleep. During each testing session, the following assessments were conducted: Epworth sleepiness scale (ESS), simple reaction time (SRT), shooting accuracy (SA), rifle disassembling and reassembling (DAS), and countermovement jump height (JH). Heart rate (HR) was continuously monitored during the SA task, and a rating of perceived exertion (RPE) was obtained during the JH task. At each time point, tests were performed twice, either with AI or without AI (CON), in a counterbalanced order. There was no condition × time interaction in any test, but there was faster SRT (1.6%; $p = 0.007$) without increasing the number of errors, higher JH (1.5%; $p = 0.005$), lower RPE (9.4%; $p < 0.001$), and higher HR (5.0%; $p < 0.001$) after using AI compared to CON regardless of TSD. However, neither SA nor DAS were affected by AI or TSD ($p > 0.05$). Independent of AI, the SRT was slower (3.2-9.3%; $p < 0.001$) in the mornings (-24, +8) than in the evening (-12), JH was higher (3.0-4.7%, $p < 0.001$) in the evenings (-12, -36) than in the mornings (0, -24, +8), and RPE was higher (20.0-40.1%; $p < 0.001$) in the sleep-deprived morning (-24) than all other timepoints (0, -12, -36, +8). Furthermore, higher ESS (59.5-193.4%; $p < 0.001$) was reported at -24 and -36 than the rest of the timepoints (0, -12, and + 8). Although there were detrimental effects of TSD, the usage of AI did not reduce those adverse effects. However, regardless of TSD, AI did result in a short-term increase in HR, improved SRT without affecting the number of errors, and improved JH while concurrently decreasing the RPE. No changes, yet, were observed in SA and DAS. These results suggest potential applications of AI in specific military scenarios, regardless of the duration of sleep deprivation.

Keywords: Army; Soldiers; Sleep Loss; Ergogenic Aids; Tests

8.1. Introduction

Sleep is a crucial, yet frequently undervalued biological process that can impact the health and combat readiness of military personnel (Thompson et al., 2017). Although healthy adults need an average of 7.5 to 8.5 hours of sleep per day (Van Dongen et al., 2003; Wehr et al., 1993), self-reported data indicates that military personnel in various branches of service obtain less sleep (Meadows et al., 2018), which may have a detrimental effect on the ability to perform military duties efficiently (Heaton et al., 2014). Furthermore, a considerable proportion of military personnel is confronted with scenarios where they are required to carry out tasks incessantly and for up to 24 hours (Department of the Army, 2013). In situations of military operational necessities such as overnight duty, prolonged operations, or direct ground combat, some soldiers may endure a lack of sleep that can surpass 24 hours, a condition known as total sleep deprivation (Reynolds & Banks, 2010).

Research suggests that total sleep deprivation can decrease blood flow velocity in the middle cerebral artery (Csipo et al., 2021), a major blood vessel that supplies the brain with oxygenated blood. This decrement has been shown to contribute to various cognitive and behavioral impairments, such as fatigue and impaired sustained attention and reaction time (Csipo et al., 2021). Moreover, extant research has indicated that manual dexterity, a critical component of tasks like shooting and firearms handling, may be negatively impacted by total sleep deprivation. Previous investigations have reported that the absence of sleep for a period from 24 to 72 hours can lead to a reduction in shooting accuracy, ranging from 13% to 37% (Dąbrowski et al., 2012; Tharion et al., 2003). Additionally, another study (Hirkani & Yogi, 2017) has demonstrated that even a single night of total sleep deprivation may have deleterious effects on manual dexterity and hand-eye coordination, resulting in a decrease in performance by 32%.

In addition to the detrimental effects on cognitive functioning and perceptual-motor skills, research has demonstrated that total sleep deprivation also impairs short-term, high-intensity exercise output. Previous research has demonstrated that total sleep deprivation lasting for 24 hours can lead to a reduction of 2% to 10% in 15-meter sprint speed (Dąbrowski et al., 2012; Skein et al., 2010; Tomczak, 2015). Moreover, after 36 hours of total sleep deprivation, short-term maximal anaerobic performance has been reported to decrease by 5% (Souissi et al., 2013). Given that soldiers frequently need to perform intense, short-term movements such as sprinting across a battlefield or traversing obstacles in various terrain conditions, a decline in this type of performance may negatively impact a soldier's survival and effectiveness in combat. Since the potential risks and high-stakes nature of military service, the detrimental effects of impaired sleep on cognitive and physical functioning are of significant concern. As such, ergogenic aids, which can alleviate these negative effects by promoting alertness and augmenting physical performance, are of interest to military (Harman et al., 2008).

One of the most widely used ergogenic aid (stimulant) consumed by military personnel is caffeine (Crawford et al., 2017). According to prior research, moderate doses of caffeine (approximately 200-300 milligrams) have been found to sustain cognitive functioning, such as alertness and attention (Crawford et al., 2017; McLellan et al., 2016) or enhance physical performance (McLellan et al., 2016) during sleep deprivation. However, the use of caffeine cannot serve as a replacement for regular sleep. Excessive caffeine consumption can further disrupt sleep patterns, particularly if consumed within six hours prior to bedtime (Good et al., 2020; Wesensten et al., 2011). Furthermore, the potential positive effects of caffeine are likely to manifest between 10 (Gonzalez et al., 2011) to 60 minutes (Graham, 2001) after consumption, which may not be sufficient if immediate assistance is required. Therefore, fast-acting forms of ergogenic aids could be beneficial in such circumstances.

An example of a fast-acting ergogenic aid are ammonia inhalants (AI), which are commonly used as a fast-acting "pre-workout stimulant", whereby users hope for rapid improvements in vigilance and short-term high-intensity physical performance (Malecek & Tufano, 2021). The putative effect of arousal via AI inhalation is believed to be caused by irritation of the respiratory passages that may subsequently trigger the adrenergic receptors in peripheral tissue, resulting in the release of norepinephrine, causing an increase in cardiac output, respiratory rate (Marshall, 1982) and an increase in blood flow velocity in the middle cerebral artery (Perry et al., 2016).

For example, prior research has documented an immediate increase in heart rate (Campbell et al., 2022; Perry et al., 2016) and a concurrent increase in middle cerebral artery blood flow velocity (Perry et al., 2016), following the inhalation of AI, indicating a transient cerebrovascular vasodilation effect that persisted for 15-30 seconds. Despite the absence of evidence indicating that AI inhalation has an impact on maximal muscular strength or endurance (Malecek & Tufano, 2021), some evidence suggests that AI may enhance alertness (Campbell et al., 2022), perceived performance (Campbell et al., 2022), explosive strength during isometric muscle actions (Bartolomei et al., 2018), as well as repeated anaerobic power performance when athletes were already fatigued (R. R. Rogers et al., 2022; Secrest et al., 2015). However, no effects were observed in dynamic "real-world" movements such as jump height (Campbell et al., 2022) or sprint time (Archer et al., 2017). In addition to that, it is important to note that while AI are widely prevalent among strength-based athletes, there is currently a significant gap in the literature regarding their specific effects on cognitive and physical performance (Malecek & Tufano, 2021).

Therefore this study aims to examine the effectiveness of AI in countering the effects of total sleep deprivation on cognitive and physical performance tests relevant to military personnel. We predict that soldiers will experience decreased cognitive and physical performance with prolonged total sleep deprivation. However, it is expected that the utilization of AI may mitigate these negative effects.

8.2. Methods

8.2.1. Participants

Eighteen healthy male cadets (24.1 ± 3.0 years, 181.5 ± 6.3 cm, 79.3 ± 8.3 kg, 4.0 ± 0.9 total years of service, all measurements reported as mean ± SD) serving at the Military department of Charles University participated in this study. The cadets were selected (Q1, 2021) primarily due to their homogenous and synchronized daily cycle based on mandatory morning lineups and the University program. Eligible participants were required to have passed an annual physical fitness test and medical checkup within the last year, have at least two years of active-duty service experience, report a high level of comfort handling firearms, be non-smokers, and currently not working shiftwork or taking medications known to interfere with sleep, cognitive or physical performance. Prior to the study onset, all participants were fully informed about the experimental design and potential risks associated with participation, and provided written informed consent in accordance with the Declaration of Helsinki. The study was also approved by the Institutional Review Board of the National Institute of Mental Health in the Czech Republic (reference number 176/20), ensuring adherence to ethical standards and guidelines.

8.2.2. Experimental Design

Data from this study are part of a broader research project aimed at investigating the effects of different light conditions on cognitive and physiological performance during periods of total sleep deprivation. We used a crossover randomized controlled trial design with within-subject repeated-measures to assess the effects of ~36 hours of total sleep deprivation and acute ammonia inhalation on occupationally relevant military tasks in military personnel.

Participants reported to the Sleep and Chronobiology laboratory (National Institute of Mental Health) on Thursday evening after a standardized dinner at ~1800 h. They then completed a series of questionnaires addressing psychological and physiological health, which were followed by a general familiarization of the layout of the facility (i.e., location of the bathrooms, testing stations, etc.). During this familiarization, the participants were also familiarized with the specific testing procedures and practiced each of the required tasks.

The actual testing protocol began with a night of uninterrupted sleep from ~2200 h to ~0630 h. Participants then underwent 5 identical testing sessions from every ~0730-0930 h in the morning and ~1900-2100 h in the evening. The first test occurred in the morning after the full night of baseline sleep (0 h) and again after 12 hours (-12 h), 24 hours (-24 h), and 36 hours of total sleep deprivation (-36 h) followed by additional testing session after 8 hours (from 2230-0630 h) of recovery sleep (+8 h). During total sleep deprivation, participants were not allowed to sleep and were kept awake in a common room by passive means, such as playing board games, watching television and reading books while under constant supervision of the research team. Furthermore, the participants were subjected to a constant ambient room light for the entire duration of total sleep deprivation period.

Participants were administered a standardized sleepiness scale and underwent simple reaction time testing, handgun shooting accuracy protocol, a rifle disassembly and reassembly protocol, and countermovement jump testing at each testing session. Participants performed each individual test twice at each testing period, either with AI (AI) or without AI (CON), in randomized order (Figure 5) and separated by 2 minutes of rest (in order to minimize any potential carryover effects of the AI) (Perry et al., 2016).

For all AI trials, a capsule containing 0.3 mL of AI (composed of 15% of ammonia and 35% of alcohol) (Dynarex, 2020) was used according to the manufacturer's instructions (Dynarex Corporation, Orangeburg, NY). When the ammonia fumes were released, researcher immediately held the capsule under the participant’s nose to inhale until a voluntary withdrawal reflex was observed (Perry et al., 2016).

During the entire study protocol, participants received personalized daily food rations consisting of standard 'ready to eat' meals commonly used in the Czech military. One week before the experiment, participants’ body composition was measured (using air displacement plethysmography; Bod Pod Body Composition System; Life Measurement Instruments, Concord, CA), and the total daily energy expenditure was derived from the estimated resting metabolic rate and application of an "active" physical activity factor of 1.6 (Conkright et al., 2021) to the individual caloric requirements. In addition, each participant was allowed ad libitum water consumption. Breakfast was consumed at ~0930 h, lunch at ~1230 h, and dinner at ~1730 h, each day (Figure 6). Additionally, all forms of stimulants were prohibited 72 hours before and during the testing protocol.

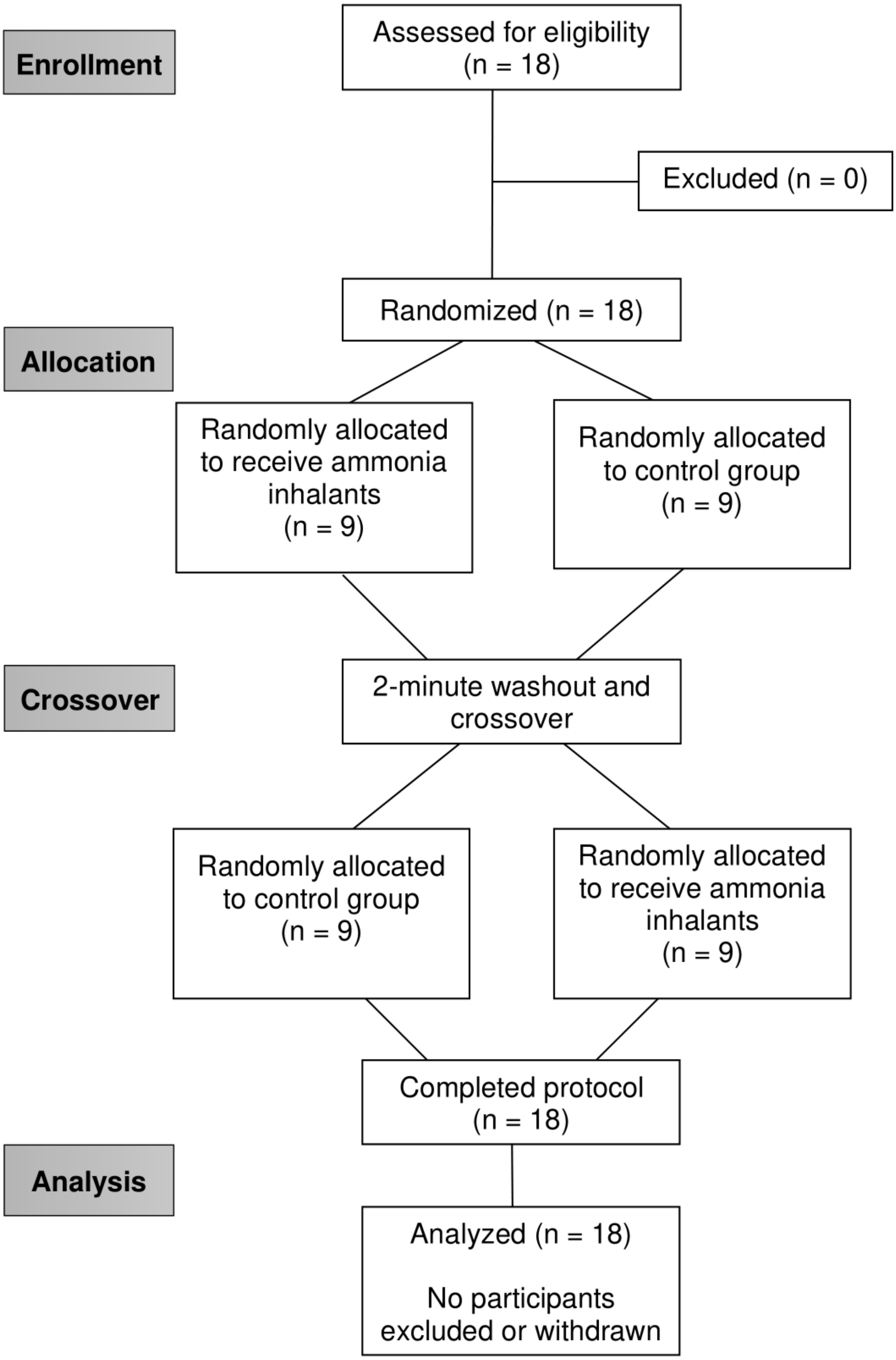

Figure 5. Flow diagram for crossover trial of each testing period either with ammonia inhalants or without (control group).

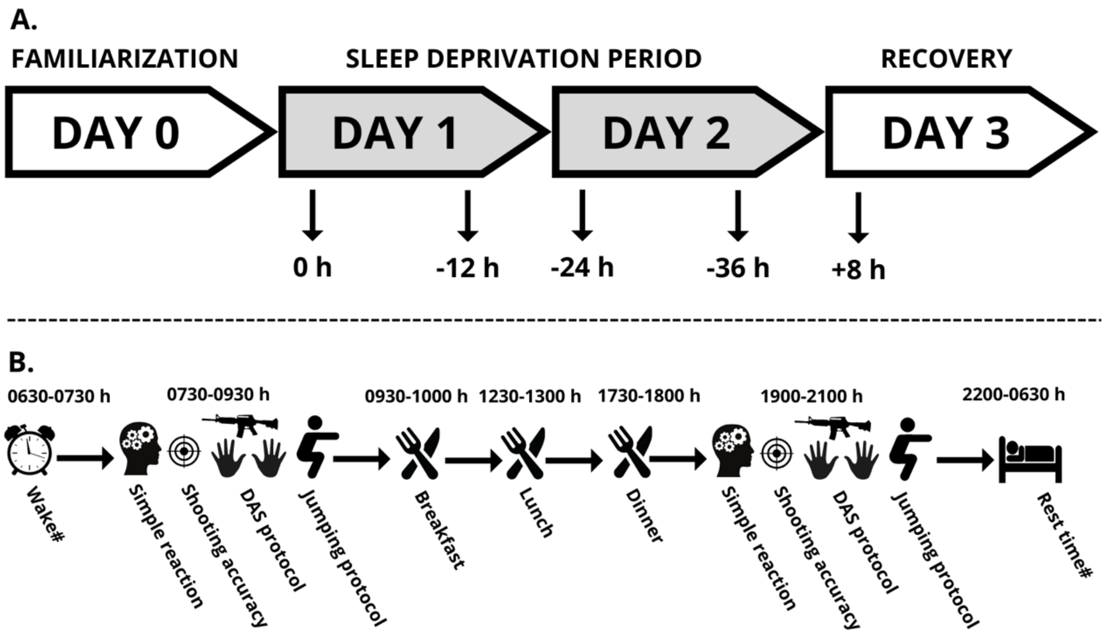

Figure 6. Overview of the study protocol. **(A)** Schedule for days 0–3 of the study, with testing session times (indicated by arrows) at baseline after a full night of sleep (0 h), after 12 (-12 h), 24 (-24 h) and 36 (-36 h) hours of total sleep deprivation followed by 8 (+8 h) hours of recovery sleep. **(B)** Daily timeline of the study. DAS protocol = rifle disassembly and reassembly protocol; #During the total sleep deprivation period between days 1 and 2, participants were not allowed to sleep. Sleep hours on nights leading into day 1 and 3 were from 2200 h to 0630 h.

8.3. Measurements

8.3.1. Epworth Sleepiness Scale

We used Epworth Sleepiness Scale (ESS) translated into Czech. It is a self-administered eight-item questionnaire and takes two to three minutes to complete (Boyes et al., 2017). The questionnaire presents daily lifestyle activities (i.e. reading, watching TV etc.) and participants rate their current self-perceived likelihood of dozing off in each situation, from: “would never doze” (0) to “high chance of dozing” (3). The ESS provides a cumulative score between 0 and 24, with higher numbers indicating greater sleepiness (Appendix 11).

8.3.2. Simple reaction time

A simple reaction time test was used to assess the speed of responses to visual stimuli (Jarraya et al., 2013). The evaluation of reaction time was performed using the Psychology Experiment Building Language (PEBL Version 2.0) software (Mueller &

Piper, 2014). The test consists of instantaneous responses to a visual stimulus by pressing a spacebar key on a laptop's keyboard as quickly as possible when a visual stimulus (white letter X in the middle of the black screen) appears. In the test, 50 trials of stimuli were presented with an interstimulus interval that randomly varied between 250 ms and 2500 ms. Each participant completed four tests (each time two, either with AI or CON, in randomized order) with 2 minutes of inter-test rest. The simple reaction time data obtained were inspected according to pre-determined criteria, which excluded trial executions that were deemed incorrect due to a reaction time shorter than 150 ms or longer than 3000 ms. The mean reaction time (measured in milliseconds) and the number of incorrect trial executions were used as the variables in the subsequent statistical analysis.

8.3.3. Shooting protocol

A laser-based simulator system (LASRX, Plano, TX & Beatrice, NE) with an infrared laser handgun (SIRT 110, Next Level Training, Ferndale, WA) was used (iron sights were used for aiming) to assess handgun shooting accuracy (Malecek et al., 2023). Each testing period included two trials of the laser-based handgun shooting protocol (either AI or CON, in randomized order). All trials were performed in the standardized isosceles high-ready stance position (i.e., feet parallel at shoulder-width with both arms extended, holding the handgun with outstretched arms and in front at eye level) (Lewinski et al., 2015). For this study, a real-weight mock-up of the Czech military standard issue Glock 17/22 handgun was used (all participants were familiar with the handgun from their active service). Participants wore over-ear headphones during all testing procedures to hear the software command to start shooting and the simulated shooting blasts when pulling the trigger. A 20 cm circular target was placed on a blank wall 4 meters in front of the participants to simulate a standard-issue 50 cm target 10 meters away for the laser-based handgun shooting protocols (the adjusted size of target was chosen due to limited room dimension available in the research facility). Each trial was separated by 2 minutes (the order of AI and CON were randomized among the participants). For the testing, participants fired 10 shots, aiming to hit the middle of the circular target (a bullseye hit was worth 10 points, and 1 point was deducted for every 1 cm region away from the bullseye, resulting in a maximum score of 100 points). Participants were instructed to try to shoot as accurately as possible within a maximum time limit of 1-minute per trial. The sum of points from each trial was recorded for future analyses.

Additionally, all participants wore a chest strap heart rate monitor (Polar Electro Inc., Model H10, Lake Success, NY, USA) during the shooting protocol. Baseline heart rate data were derived as a mean of heart rate from 2 minutes immediately preceding the start of the shooting trial. Heart rate (bpm) was then continuously monitored during all sessions of shooting protocol. After baseline testing, heart rate data were averaged in 15 seconds bins (0-15, 15-30, 30-45, 45-60) for one minute immediately following the AI and CON trials. The mean of these bins from each trial was used in subsequent analysis (Perry et al., 2016).

8.3.4. Jumping protocol

We used unloaded countermovement jump (CMJ), one of the most common and straightforward strategies to monitor short-term neuromuscular performance in tactical populations (Merrigan et al., 2020). Each CMJ session included 2 sets (AI and CON in a randomized order) of 3 maximal effort CMJs with 2 min of inter-set standing rest. The researcher verbally instructed and encouraged the participants to jump as high as possible on each jump. All CMJs were performed with wooden dowel (~ 0.5 kg) as a mock barbell placed across the participant's upper back mimicking a regular back squat. A linear position transducer (GymAware Power Tool; Kinetic Performance Technologies, Canberra, Australia) was attached to both sides of a dowel to measure the performance. The depth of the CMJ depth was self-selected. Participants wore the same sports t-shirts, shorts and shoes during each test period. The mean of the 3 jump heights (cm) was calculated for each condition at each test session.

In addition, the rating of perceived exertion (RPE) was recorded during the CMJ testing using a CR-10 scale (Appendix 12) to evaluate RPE scores after each set of CMJ (Day et al., 2004). RPE is a frequently used marker of exercise intensity typically used for monitoring during exercise tests to complement other intensity measures (Eston, 2012).

8.3.5. Rifle disassembly and reassembly protocol

The protocol for disassembling and reassembling a military-standard issue assault rifle (specifically the Czech vz. 58 assault rifle) was selected to assess changes in manual dexterity as it is representative tasks that soldiers may encounter in field operations (Kryskow et al., 2013). During the protocol, participants were tasked to disassemble and reassemble a rifle consisting of 8 parts as fast as possible. Prior to the task's onset, standing participants were instructed to place their hands behind their backs and wait for the researcher's "start" command, after which they attempted to disassemble the rifle as quickly as possible. After a two-minute break, during which participants organized the rifle parts on a table, they then proceeded to reassemble the rifle under the same instruction, and the time was recorded. During the reassembling, the final step was conducting a successful "rifle function check". The time for completion of the task was measured using a handheld stopwatch and recorded on a digital camera for possible corrections.

Each participant completed two trials of rifle disassembly and reassembly (AI and CON, in a randomized order) with two minutes of rest between conditions. The performance measure used in this study was the sum of the disassembly and reassembly time in seconds for each condition (AI and CON).

To assess the reliability of the task, participants familiarize themselves with the disassembly and reassembly protocol before the study begins. They repeatedly performed the protocol for three consecutive days, one week before participating in the study. Their performance showed sufficient reliability (data and reliability analysis of the familiarization period can be found in the supplementary materials).

During the testing protocol, data were recorded for 14 of the 18 participants. The remaining four participants were unable to participate in the familiarization due to service duties and were thus excluded from the respective subsequent analysis.

8.3.6. Statistical analysis

Statistical analyses were conducted using JASP (version 0.16.2, 2022) (Jasp Team, 2022). Parametric tests were performed once the normality assumptions were verified using the Shapiro-Wilk W test. Data were analyzed using a two-way repeated measures ANOVA (2 conditions: [AI, CON] × 5 time: [0 h, -12 h, -24 h, -36 h, +8 h]). Heart rate values were analyzed using a three-way repeated measures ANOVA (2 conditions: [AI, CON] × 5 time: [0 h, -12 h, -24 h, -36 h, +8 h] × 4 time spans: [0-15, 15-30, 30-45, 45-60 sec]). Lastly, the heart rate percentage difference values were analyzed using a one-way repeated measures ANOVA (1 condition: [heart rate percentage difference between AI and CON] × 4 time spans: [0-15, 15-30, 30-45, 45-60 sec]). Sphericity was assessed using Mauchly's W. In cases where sphericity assumptions were violated, a Greenhouse–Geisser adjustment was applied. When the ANOVA tests demonstrated a statistically significant condition × time (× time spans or × percentage difference) interaction or a statistically significant main effect for condition, time, time spans, or percentage difference, post-hoc comparisons (with Bonferroni correction) of the mean differences were performed. The variance explained by each ANOVA model is reported in η^2, and the mean difference effect sizes are reported as Cohen's d with 95% confidence intervals [LL, UL].

8.3.7. Sensitivity analysis

Due to the limited number of cadets at the Military department and the limited capacity of the sleep laboratory (6 participants per measurement at one time), the maximum number of participants was limited to 18. We performed sensitivity analyses to observed effects in our tests using G*Power (version 3.1.). For the within-factor differences and within-between interaction in repeated measures ANOVAs with $\alpha = 0.05$, $\beta = 0.8$, N = 18, 2 conditions, and 5 testing sessions, we reached a sensitivity to observe a Cohen's f = 0.267, which corresponds to $\eta^2 = 0.066$ (default values of correlation and sphericity of 0.5 and 1, respectively, were used).

For the post-hoc comparisons, we calculated the sensitivity for two-tailed T-tests of dependent means with matched pairs. With a Bonferroni-adjusted α of 0.01 (calculated as 0.05 divided by the number of comparisons, 5 in our case), $\beta = 0.8$ (J. Cohen, 1992), N = 18, we reach sensitivity to observe an effect size (Cohen's d) of 0.894.

8.3.8. Registration and supplementary materials

Associated dataset and JASP outputs for all performed analyses are available at the Open Science Framework [OSF] repository (URL: https://osf.io/3rj84/; DOI 10.17605/OSF.IO/3RJ84).

The authors confirm that all ongoing and related trials for this drug/intervention are registered, including registration on ClinicalTrials.gov under the identifier number NCT05868798. The project was not preregistered as it was not a common practice in the field at the time of the project's development and data collection.

8.4. Results

8.4.1. Simple reaction time and incorrect trials

In simple reaction times, we observed statistically significant main effect of time ($F_{2.30,\ 6291.19} = 4.99$, $p = 0.009$, $\eta^2 = 0.19$, with Greenhouse-Geisser correction). In subsequent post-hoc comparisons, the simple reaction time was statistically significantly slower at -12 compared to -24 (mean difference = 26.74 ms, p_{bonf} = 0.002, Cohen's d = 1.05 [0.15, 1.94]) and + 8 (mean difference = -18.04 ms, p_{bonf} = 0.007, Cohen's d = 0.70 [-1.51, -0.10]) (Figure 7A).

There was also statistically significant main effect of condition ($F_{1, 1060.42}$ = 9.40, p = 0.007, η^2 = 0.014), demonstrating that application of AI reduced simple reaction time (mean difference = 4.85 ms, p_{bonf} = 0.007, Cohen's d = 0.19 [0.04, 0.34]). We observed no statistically significant condition × time interaction ($F_{4, 113.71}$ = 0.83, p = 0.50, η^2 = 0.006) (Figure 7A).

Though there was no statistically significant main effect of time ($F_{2.262, 2.619}$ = 1.437, p_{bonf} = 0.250, η^2 = 0.051, with Greenhouse-Geisser correction) for incorrect trials execution, there was statistically significant main effect of condition ($F_{1, 3.200}$ = 6.800, p_{bonf} = 0.018, η^2 = 0.028), demonstrating that using AI reduced the amount of incorrect trials (mean difference = 0.267, p_{bonf} = 0.018, Cohen's d = 0.267 [0.04, 0.50]). There was no statistically significant condition × time interaction ($F_{4, 0.742}$ = 1.952, p_{bonf} = 0.112, η^2 = 0.026) (Figure 7B).

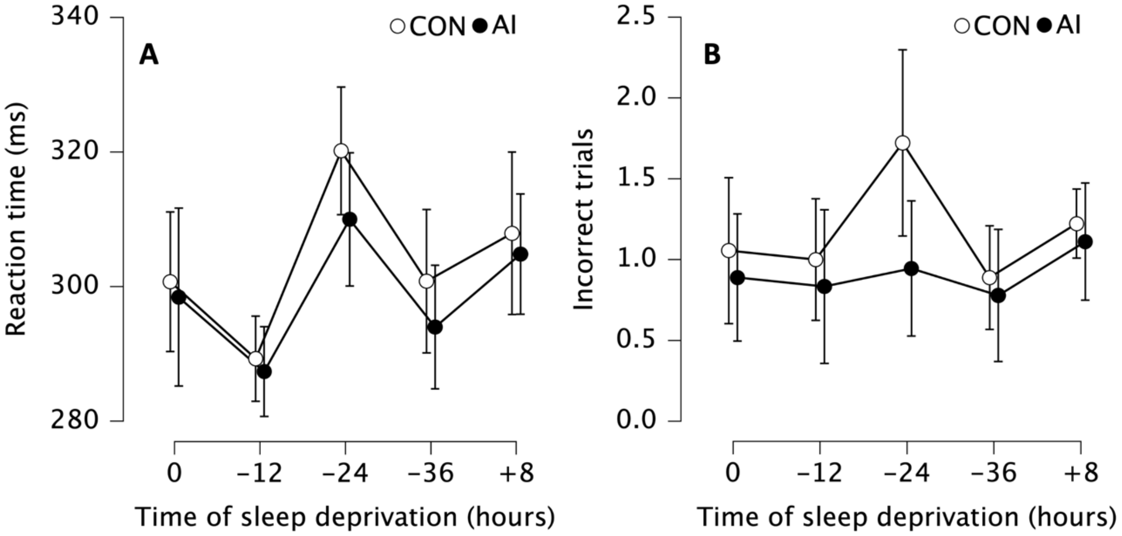

Figure 7. (A) Mean simple reaction time and **(B)** number of incorrect trials without (CON, white) and with ammonia inhalants (AI, black). Data are presented as mean (circles) and error bars represent their 95% confidence interval (LL, UL) at baseline (0 h), after 12 (-12 h), 24 (-24 h) and 36 (-36 h) hours of total sleep deprivation followed by 8 (+8 h) hours of recovery sleep.

8.4.2. Shooting accuracy

For shooting accuracy, we observed no statistically significant main effect of time ($F_{2.834, 35.844} = 1.755$, $p_{bonf} = 0.171$, $\eta^2 = 0.045$, with Greenhouse-Geisser correction) or main effect of condition ($F_{1, 27.222} = 1.529$, $p_{bonf} = 0.233$, $\eta^2 = 0.012$). There was also no statistically significant condition × time interaction ($F_{4, 2.208} = 0.182$, $p_{bonf} = 0.947$, $\eta^2 = 0.004$).

8.4.3. Heart rate during shooting

We observed statistically significant main effect of time ($F_{4, 2959.875} = 8.515$, $p_{bonf} < 0.001$, $\eta^2 = 0.126$) on heart rate during shooting. In subsequent post-hoc tests, the heart rate was statistically significantly slower at 0 than at -12 (mean difference = 9.29 bpm, $p_{bonf} = 0.004$, Cohen's d = 0.84 [0.15, 1.53]) and also slower at -24 compare to -12 (9.75 bpm, $p_{bonf} = 0.001$, Cohen's d = 0.88 [0.17, 1.59]) and -36 (5.63 bpm, $p_{bonf} = 0.018$, Cohen's d = 0.51 [0.09, 1.11]) (Figure 8A).

There was statistically significant main effect of condition ($F_{1, 2022.301} = 52.844$, $p_{bonf} < 0.001$, $\eta^2 = 0.021$) demonstrating that using AI increased heart rate (mean difference = 2.99 bpm, $p_{bonf} < 0.001$, Cohen's d = 0.272 [0.15, 0.39]). There was also statistically significant main effect of time span ($F_{2.277, 17517.031} = 197.227$, $p_{bonf} < 0.001$, $\eta^2 = 0.423$, with Greenhouse-Geisser correction), demonstrating the highest heart rate at 0-15 time span compare to baseline (mean difference = 20.01 bpm, $p_{bonf} < 0.001$, Cohen's d = 1.813 [0.80, 2.82]), 15-30 (mean difference = 5.21 bpm, $p_{bonf} < 0.001$, Cohen's d = 0.472 [0.14, 0.81]), 30-45 (mean difference = 8.72 bpm, $p_{bonf} < 0.001$, Cohen's d = 0.79 [0.31, 1.27]), and 45-60 (mean difference = 11.21 bpm, $p_{bonf} < 0.001$, Cohen's d = 1.01 [0.42, 1.61]).

There was also a statistically significant condition × time span interaction ($F_{4, 212.765} = 22.593$, $p_{bonf} < 0.001$, $\eta^2 = 0.009$) (Figure 8B), demonstrating that after using AI we observed the highest heart rate at AI 0-15 compare to baseline (mean difference = 22.95 bpm, $p_{bonf} < 0.001$, Cohen's d = 2.08 [0.68, 3.47]), AI 15-30 (mean difference = 6.13 bpm, $p_{bonf} < 0.001$, Cohen's d = 0.55 [0.09, 1.02]), AI 30-45 (mean difference = 10.28. bpm, $p_{bonf} < 0.001$, Cohen's d = 0.93 [0.26, 1.60]), and AI 45-60 (mean difference = 13.00 bpm, $p_{bonf} < 0.001$, Cohen's d = 1.18 [0.35, 1.99]).

Without the use of AI we observed highest heart rate in CON 0-15 compare to baseline (mean difference = 17.07 bpm, $p_{bonf} < 0.001$, Cohen's d = 1.54 [0.49, 2.60]), CON 15-30 (mean difference = 4.29 bpm, $p_{bonf} < 0.001$, Cohen's d = 0.39 [0.01, 0.77]), CON 30-45 (mean difference = 7.16 bpm, $p_{bonf} < 0.001$, Cohen's d = 0.65 [0.14, 1.16]), and CON 45-60 (mean difference = 9.43 bpm, $p_{bonf} < 0.001$, Cohen's d = 0.85 [0.30, 1.45]).

Additionally, all heart rates in same time spans after AI administration were higher compared to CON, whereas AI 0-15 was higher than CON 0-15 (mean difference = 5.88 bpm, $p_{bonf} < 0.001$, Cohen's d = 0.53 [0.13, 0.93]), AI 15-30 was higher than CON 15-30 (mean difference = 4.04 bpm, $p_{bonf} < 0.001$, Cohen's d = 0.36 [0.53, 0.68]), AI 30-45 was higher than CON 30-45 (mean difference = 2.76 bpm, $p_{bonf} < 0.001$, Cohen's d = 0.25 [0.01, 0.51]), AI 45-60 was higher than CON 45-60 (mean difference = 2.31 bpm, $p_{bonf} < 0.001$, Cohen's d = 0.21 [0.03, 0.45]) (Figure 8B).

There was no statistically significant condition × time interaction ($F_{4, 17.423} = 0.803$, $p_{bonf} = 0.528$, $\eta^2 = 0.0007$). There was also no statistically significant time × time span interaction ($F_{5.113, 150.320} = 1.848$, $p_{bonf} = 0.110$, $\eta^2 = 0.008$) and condition × time × time span interaction ($F_{7.337, 8.906} = 0.590$, $p_{bonf} = 0.771$, $\eta^2 = 0.0006$).

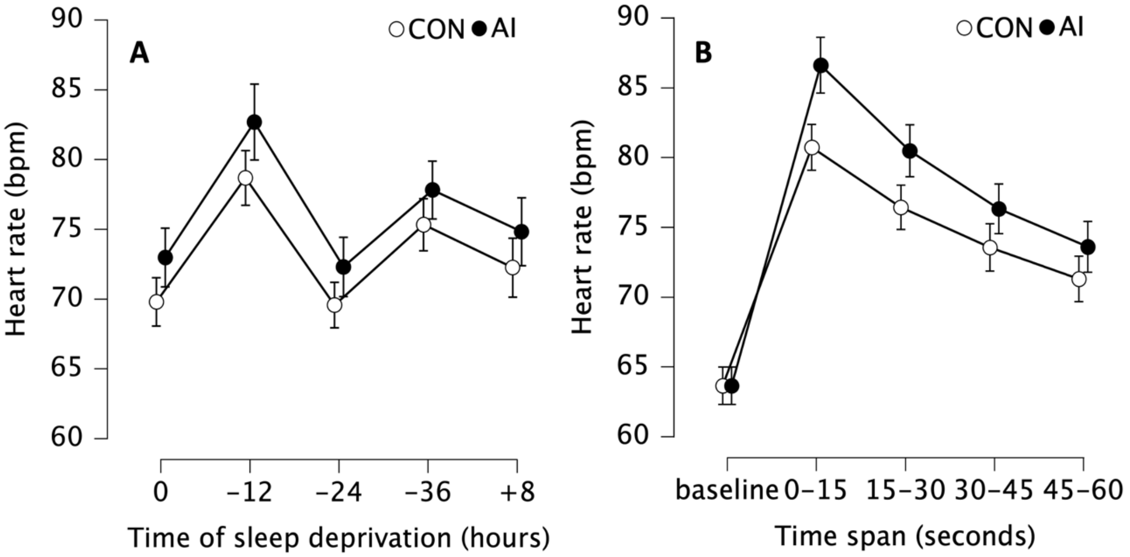

Figure 8. (A) Mean heart rate (bpm) without (CON, white) and with ammonia inhalants (AI, black). Data are presented as mean (circles) and error bars represent their 95% confidence interval (LL, UL) at baseline (0 h), after 12 (-12 h), 24 (-24 h) and 36 (-36 h) hours of total sleep deprivation followed by 8 (+8 h) hours of recovery sleep. **(B)** Mean heart rate (bpm) without (CON, white) and with ammonia inhalants (AI, black). Data are presented as mean (circles) and error bars represent their 95% confidence interval (LL,

UL) as an average of 15-second time spans (0-15, 15-30, 30-45, and 45-60) during shooting trials regardless of sleep deprivation.

Additionally, there was statistically significant main effect of percentage difference between AI and CON during the same time spans ($F_{3.\ 61.833} = 8.776$, $p_{bonf} < 0.001$, $\eta^2 = 0.340$), where we observed greater percentage difference at 0-15 than 30-45 (mean difference = 3.67 %, $p_{bonf} = 0.012$, Cohen's d = 1.02 [0.18, 1.85]) and 45-60 (mean difference = 4.08 %, $p_{bonf} = 0.005$, Cohen's d = 1.13 [0.27, 2.00]) (Figure 9).

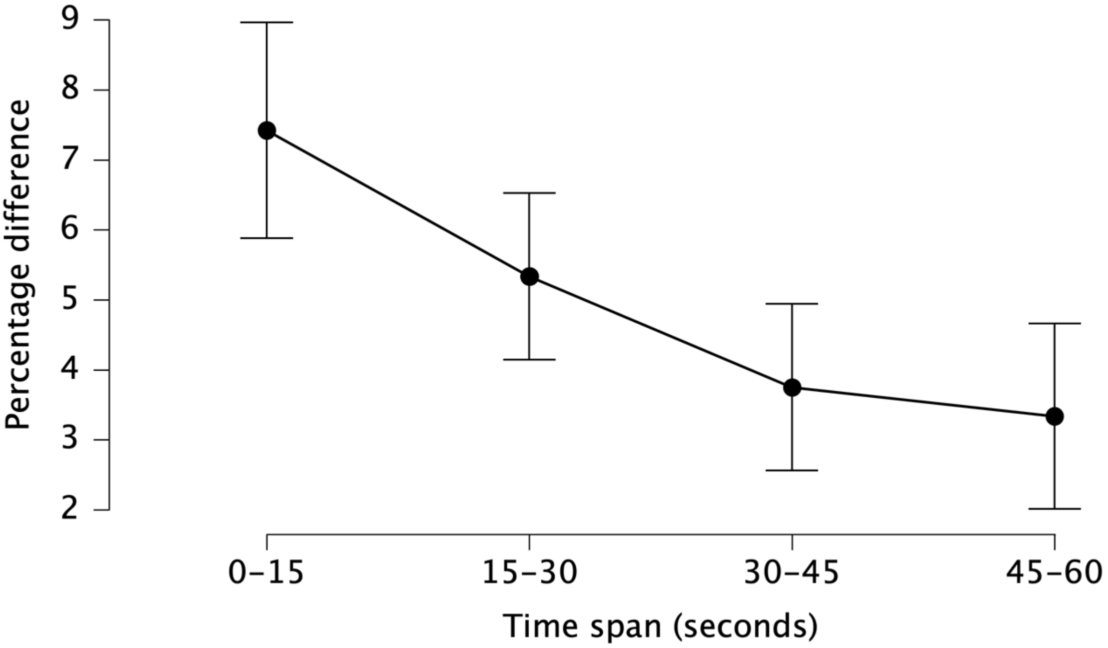

Figure 9. Mean heart rate percentage difference (between CON and AI at each 15-second time span). Data are presented as mean (circles) and error bars represent their 95% confidence interval (LL, UL) at the average of 15-second time spans (0-15, 15-30, 30-45, and 45-60) during shooting trials regardless of sleep deprivation.

8.4.4. Countermovement jump height

In the case of jump height, we observed statistically significant main effect of time ($F_{4,\ 29.447} = 8.070$, $p_{bonf} < 0.001$, $\eta^2 = 0.035$). In subsequent post-hoc tests, the jump height was statistically significantly higher in the evenings compare to the mornings. Particularly -12 was higher than 0 (mean difference = 1.88 cm, $p_{bonf} = 0.015$, Cohen's d = 0.33 [0.22, 0.65]), -24 (mean difference = 1.33 cm, $p_{bonf} = 0.010$, Cohen's d = 0.24 [-0.05, 0.52]), +8 (mean difference = 1.35 cm, $p_{bonf} = 0.046$, Cohen's d = 0.24 [-0.05, 0.53]), and also -36 was higher compare to 0 (mean difference = 2.03 cm, $p_{bonf} = 0.006$, Cohen's d = 0.36 [-0.04, 0.67]) and -24 (mean difference = 1.50 cm, $p_{bonf} = 0.014$, Cohen's d = 0.26 [-0.28, 0.56]) (Figure 10).

There was also a statistically significant main effect of condition ($F_{1, 18.142} = 10.576$, $p_{bonf} = 0.005$, $\eta^2 = 0.035$), demonstrating that jump height after AI administration was higher (mean difference = 0.64 cm, $p_{bonf} = 0.005$, Cohen's d = 0.11 [0.03, 0.20]) compared to CON. There was no statistically significant condition × time interaction ($F_{4, 0.437} = 0.292$, $p_{bonf} = 0.882$, $\eta^2 = 0.003$) (Figure 10).

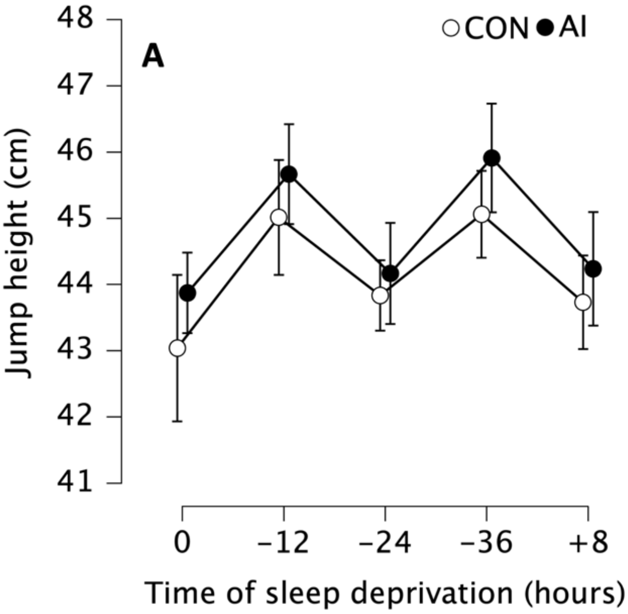

Figure 10. Mean countermovement jump height without (CON, white) and with ammonia inhalants (AI, black). Data are presented as mean (circles) and error bars represent their 95% confidence interval (LL, UL) at baseline after a full night of sleep (0 h), after 12 (-12 h), 24 (-24 h) and 36 (-36 h) hours of total sleep deprivation followed by 8 (+8 h) hours of recovery sleep.

8.4.5. Rating of perceived exertion and Epworth sleepiness scale

We observed statistically significant main effect of time ($F_{2.844, 10.030} = 10.478$, $p_{bonf} < 0.001$, $\eta^2 = 0.282$, with Greenhouse-Geisser correction) indicating that soldiers reported highest RPE at -24 compare to 0 (mean difference = 1.11, $p_{bonf} < 0.001$, Cohen's d = 1.19 [0.32, 2.06]), -12 (mean difference = 1.03, $p_{bonf} = 0.010$, Cohen's d = 1.10 [-0.26, 1.93]), and +8 (mean difference = 0.86, $p_{bonf} = 0.002$, Cohen's d = 0.92 [0.14, 1.70]).

There was also statistically significant main effect of condition ($F_{1, 3.472} = 17.220$, $p_{bonf} < 0.001$, $\eta^2 = 0.034$), demonstrating that soldiers reported higher RPE at CON compared to after usage of AI (mean difference = 0.28, $p_{bonf} < 0.001$, Cohen's d = 0.30

[0.11, 0.48]). There was no statistically significant condition × time interaction ($F_{4,\,0.264} = 0.968$, $p_{bonf} = 0.431$, $\eta^2 = 0.010$) (Figure 11A).

For ESS, we observed statistically significant main effect of time ($F_{4\,450.572} = 39.764$, $p_{bonf} < 0.001$, $\eta^2 = 0.701$), indicating a statistically significantly greater sleepiness score at -24 compare to 0 (mean difference = 11.72, $p_{bonf} < 0.001$, Cohen's d = 2.91 [1.25, 4.58]), -12 (mean difference = 9.11, $p_{bonf} < 0.001$, Cohen's d = 2.26 [0.87, 3.65]), -36 (mean difference = 3.94, $p_{bonf} = 0.018$, Cohen's d = 0.98 [0.03, 1.93]), and +8 (mean difference = 10.89, $p_{bonf} < 0.001$, Cohen's d = 2.70 [1.13, 4.28]) (Figure 11A).

Additionally, ESS was also statistically significantly greater at -36 compared to 0 (mean difference = 7.78, $p_{bonf} < 0.001$, Cohen's d = 1.93 [0.63, 3.20]), -12 (mean difference = 5.17, $p_{bonf} = 0.002$, Cohen's d = 1.28 [0.25, 2.32]), and +8 (mean difference = 6.94, $p_{bonf} < 0.001$, Cohen's d = 1.72 [0.54, 2.91]) (Figure 11B).

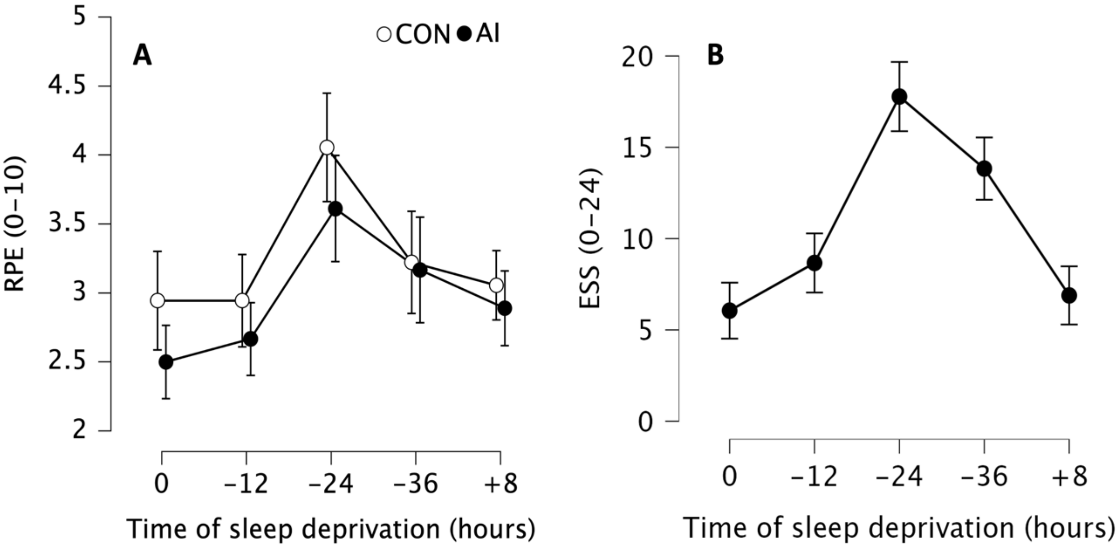

Figure 11. (A) Mean rating of perceived exertion (RPE) without (CON, white) and with ammonia inhalants (AI, black). Data are presented as mean (circles) and error bars represent their 95% confidence interval (LL, UL) at baseline after a full night of sleep (0 h), after 12 (-12 h), 24 (-24 h) and 36 (-36 h) hours of total sleep deprivation followed by 8 (+8 h) hours of recovery sleep. **(B)** Mean Epworth Sleepiness Scale (ESS) score. Data are presented as mean (circles) and error bars represent their 95% confidence interval (LL, UL) at baseline after a full night of sleep (0 h), after 12 (-12 h), 24 (-24 h) and 36 (-36 h) hours of total sleep deprivation followed by 8 (+8 h) hours of recovery sleep.

8.4.6. Rifle disassembly and reassembly protocol

For the sum of the rifle disassembly and reassembly time there was no statistically significant main effect of time ($F_{1,\,1.362} = 0.098$, $p_{bonf} = 0.759$, $\eta^2 = 0.0008$), the main effect of condition ($F_{4,\,10.825} = 0.754$, $p_{bonf} = 0.560$, $\eta^2 = 0.027$) nor condition × time interaction ($F_{4,\,11.476} = 1.046$, $p_{bonf} = 0.393$, $\eta^2 = 0.029$). Descriptions of all results can be seen in the following table (Table 4).

Table 4. Descriptive statistics of all results at baseline after a full night of sleep (0 h), after 12 (-12 h), 24 (-24 h) and 36 (-36 h) hours of total sleep deprivation followed by 8 (+8 h) hours of recovery sleep (data are presented in mean ± SD, AI = ammonia inhalants, CON = without ammonia inhalants, RPE = rating of perceived exertion, ESS = Epworth Sleepiness Scale, DAS = rifle disassembly and reassembly protocol.

			Time of total sleep deprivation				
Task	**Condition**	**N**	**0**	**-12**	**-24**	**-36**	**+8**
Simple reaction time (ms)	CON	18	300.72 ± 21.13	289.3 ± 18.40	320.17 ± 18.40	300.81 ± 18.40	307.91 ± 18.40
	AI		298.43 ± 26.40	287.38 ± 16.76	310.00 ± 30.95	293.99 ± 21.83	304.84 ± 24.36
Shooting accuracy (points)	CON	18	86.72 ± 3.33	86.72 ± 3.97	86.17 ± 4.02	85.89 ± 3.59	84.28 ± 4.66
	AI		87.00 ± 5.41	87.28 ± 3.18	87.39 ± 3.65	86.33 ± 5.55	85.67 ± 4.86
Heart rate (bpm)	CON	18	72.06 ± 8.82	81.24 ± 13.43	71.95 ± 9.72	77.39 ± 12.80	74.84 ± 11.12
	AI		76.05 ± 10.60	86.23 ± 15.84	75.38 ± 11.54	80.5 ± 12.50	78.06 ± 11.91
Jump height (cm)	CON	18	43.04 ± 5.75	45.01 ± 5.58	43.83 ± 5.64	45.06 ± 5.37	43.73 ± 4.96
	AI		43.87 ± 5.52	45.67 ± 5.82	44.17 ± 5.02	45.91 ± 5.55	44.24 ± 5.35
RPE (0-10)	CON	18	2.94 ± 1.03	2.94 ± 0.97	4.06 ± 0.78	3.22 ± 1.13	3.06 ± 0.62
	AI		2.50 ± 0.90	2.67 ± 0.94	3.61 ± 0.89	3.17 ± 1.12	2.89 ± 0.46
ESS (0-24)	x	18	6.06 ± 3.14	8.67 ± 4.63	17.78 ± 3.17	13.83 ± 4.54	6.88 ± 3.80
DAS (sec)	CON	14	33.18 ± 5.75	33.03 ± 4.95	33.69 ± 4.12	31.15 ± 3.01	31.71 ± 4.15
	AI		32.84 ± 3.71	31.71 ± 4.25	32.21 ± 2.61	31.78 ± 4.35	33.23 ± 5.02

8.5. Discussion

This study aimed to investigate the potential effect of AI usage on simple reaction time, shooting accuracy, countermovement jump height, and rifle disassembly and reassembly time during 36 h of total sleep deprivation, followed by 8 h of recovery sleep in military personnel. The principal findings of our investigation were that although both total sleep deprivation and AI affected some variables during the study protocol, AI inhalation did not have a great effect specifically during the period of sleep deprivation (i.e., there were no condition × time interactions). Nevertheless, regardless of sleep deprivation, AI use increased heart rate from 0-15 seconds after inhalation, improved simple reaction time without increasing the number of errors, and increased countermovement jump height while simultaneously decreasing rating of perceived exertion. No changes in shooting accuracy or the rifle disassembly and reassembly time after AI were observed.

8.5.1. Simple reaction time

It has been documented that prolonged periods of total sleep deprivation can impair sustained vigilance, which may negatively impact cognitive performance (Lim & Dinges, 2008, 2010) and potentially impair military readiness. The present study found that simple reaction time (SRT) was slower (3.2-9.3%) in the mornings (following 24 h of sleep deprivation, and again after 8 h of recovery sleep) as compared to the evening (following 12 h of sleep deprivation). This is partly consistent with a previous report (Goel et al., 2009) showing that when straight wakefulness exceeds 16 h (typically the end of a normal day), SRT slowed down. These already slow SRTs became even slower as wakefulness was maintained throughout the night into the early morning hours leading up to 24 h of total sleep deprivation. However, the current study observed that this phenomenon did not persist when total sleep deprivation reached a maximum of 36 h, and instead, SRT showed an improvement (5.9%) compared to the 24-h time point.

One potential explanation may be that cognitive performance is influenced by the interplay between the sleep homeostatic system (i.e., the biological drive for sleep) and the circadian rhythm (i.e., synchronization with the 24-h day/night cycle) (N. L. Rogers, 2003). These systems operate in a coordinated manner, following a 24-h sinusoidal pattern, with increased or decreased levels of fatigue depending on the time of day. As a result, total sleep deprivation can affect cognitive performance in a non-linear fashion, with the most pronounced cognitive impairments observed during the morning hours (Babkoff et al., 1991; Killgore, 2010). Our results also align with previous research (Mollicone et al., 2010), where faster SRT was reported in the evenings (after 8-12 and 32-36 h of total sleep deprivation) compared to the morning (after 24 h of total sleep deprivation). This phenomenon is also known as the "wake maintenance zone", where the desire to sleep is usually the lowest in the evenings (2 to 3 h before the start of the melatonin secretion), and it is also maintained despite the increasing drive for sleep that results from total sleep deprivation (Shekleton et al., 2013).

Even though SRT was slower in the mornings as compared to the evenings, our study aimed to determine whether using AI would affect SRT during total sleep deprivation. Partly in line with our predictions, SRT was faster following the use of AI (1.6%), but AI had no greater effect on SRT during sleep deprivation than when fully rested. Regardless of sleep deprivation, these ergogenic effects of AI are thought to be mediated by the activation of the olfactory and trigeminal nerves, which leads to the activation of the adrenergic receptor and the subsequent release of norepinephrine, resulting in an increase in cardiac output and respiratory rate (Malecek & Tufano, 2021). This effect has been shown to result in an increase in beat-to-beat middle cerebral artery blood flow velocity (Perry et al., 2016), which may be linked to enhanced cognitive performance (Pase et al., 2014).

Hence, it is plausible that the increase in alertness following the use of AI may be attributed to improved delivery of oxygenated blood to the brain (Jorgensen et al., 1992). Previous studies have indicated that decreased cerebral blood flow can impair cognitive function (Sieck et al., 2016), which may result in reduced arousal levels, ultimately leading to decreased performance (Baker et al., 2000). Considering this evidence, the enhancement of alertness (Campbell et al., 2022; R. R. Rogers et al., 2022) resulting from AI use may explain the widespread use of this stimulant. Nevertheless, it should be noted that the implications of these findings are beyond the scope of this study.

Despite this, our results suggest that the utilization of AI may be beneficial for soldiers, as it may improve their SRT without increasing the number of errors, regardless of the level of total sleep deprivation. In military contexts, where performance demands are high and have significant implications for many individuals' well-being, SRT can be a critical determinant of success or failure and may even have life-or-death consequences (Petrofsky et al., 2021). To sum up, even though it must be taken into account that the difference we observed was on the order of milliseconds, the utilization of AI may have potential applications in specific military scenarios, no matter the length of sleep deprivation.

8.5.2. Cardiovascular response

It is widely acknowledged that the autonomic nervous system influences heart rate (HR) through the discharges of the sympathetic and the parasympathetic nervous system via sympathetic and vagal innervations, respectively (Jackowska et al., 2012). However, evidence regarding the effect of total sleep deprivation on these systems, remains inconsistent (Jackowska et al., 2012). One study reported (Holmes et al., 2002) that after 30 h of total sleep deprivation, there was a decrease in cardiac sympathetic activity, but no change in parasympathetic, which contradicts the findings of another study (Zhong et al., 2005) that found increased sympathetic activity and decreased parasympathetic activity following 36 h of total sleep deprivation. Despite the inconsistent findings of previous studies, our results indicated that HR during shooting protocol was lower after 24 h of total sleep deprivation, with a slight increase after 36 h, without considering the effects of AI. These results partly align with the outcomes of laboratory studies that controlled for environmental and behavioral influences such as sleep, light and activity, reporting that 24-30 h of total sleep deprivation results in a decrease in HR in healthy young individuals (Burgess et al., 1997; Chen, 1991; Kerkhof et al., 1998). This decline in HR is typically superimposed on a 24-h rhythm (Holmes et al., 2002), which could be a potential explanation for the increase in HR after 36 h of sleep deprivation in the current study.

Nevertheless, this study also investigated the impact of AI inhalation on HR during shooting protocol while total sleep deprived. Our findings are in line with a previous study (Perry et al., 2016), which demonstrated that AI elicits a strong cardiovascular response that results in an elevation of HR compared to control trials without AI. Additionally, our results showed that this response was observed immediately after AI inhalation, with HR increasing in the first 15 seconds (7.3% compare to CON) and then gradually declining (Figure 8). That is consistent with prior reports indicating that the effect of AI on the cardiovascular system is typically short-term and may not provide a sustained ergogenic benefit beyond the initial 15 seconds of inhalation (Malecek & Tufano, 2021; Perry et al., 2016).

In sum, despite the consistency of our findings with those of the prior investigation, it is imperative to consider that the regulation of HR is predominantly controlled by the autonomic nervous system and the opposing actions of its sympathetic and parasympathetic components. This constitutes a complex system that may be impacted by various confounding factors during the prolonged experiments, as were conducted in this study, such as dietary intake, energy balance, hydration, physical activity, effect of shooting stress, temperature regulation, and psychological stress. Thus, the following potential studies should consider testing cardiovascular responses throughout the whole study period.

8.5.3. Shooting accuracy

Assuming that inhaling AIs could be associated with higher arousal (Malecek & Tufano, 2021), we had predicted that AI could attenuate the shooting accuracy decrements that would be caused by sleep deprivation (Torres & Kim, 2019). However, in the context of the present study, neither sleep deprivation nor the presence of AI (i.e., increased HR) affected shooting accuracy. The results of our study partially concur with previous research that found that the live-fire shooting accuracy of trained soldiers was not impacted by total sleep deprivation ranging from 24 to 36 h (McLellan et al., 2016; Tomczak et al., 2017). However, it has been reported that similarly extended total sleep deprivation resulted in impaired live-fire shooting accuracy among conventional military personnel and non-military trained individuals (Dąbrowski et al., 2012; McLellan et al., 2016; Tikuisis et al., 2004). This possibly suggests that more experienced shooters (with longer training experience) may be more resilient to the possible adverse effects of total sleep deprivation, which may explain the consistent shoot accuracy found in the present study.

Despite these fairly straightforward findings, some observations in our study diverged from previous findings. For example, one study shows that shooting accuracy decreased as HR increased in standing positions, but another study (Hoffman et al., 1992) suggests that low-intensity exercise, which leads to commensurate increases in HR, may initially enhance shooting accuracy before a decline is observed. Nonetheless, the different outcomes observed could be attributed to the substantial intra-individual variability in managing psychological factors, such as fatigue and stress, which may impact HR and, consequently, shooting accuracy among individuals (Vickers & Williams, 2007). Therefore, while our results agree with previous research (Tharion et al., 2003; Tikuisis et al., 2004) that also evaluated shooting accuracy using small arms simulators rather than live fire, it is crucial to acknowledge that the use of simulated weapons in evaluating shooting performance may affect the applicability of the findings to real-life military situations (Vickers & Williams, 2007).

In the present study, it is challenging to differentiate the various effects of HR as an indicator of physiological exertion or mechanical perturbation. This intra-individual variability may be attributed to the heightened mechanical impact of the heartbeat on shooting dynamics or may indicate an "over-arousal" that results in a decrease in performance, as demonstrated by the Yerkes-Dodson performance-arousal curve (Tenan et al., 2017). With all these points in mind, further research should delve into the interaction between these mechanical, physiological, and psychological factors that influence shooting more deeply to better understand the interplay of these elements. In summary, this study highlights that although AI did not improve handgun shooting accuracy, it was posed no negative effect either. It is still important to keep in mind the parameters of our study (observed effect sizes). However, the unaffected shooting accuracy, combined with the decreased SRT noted in the previous section, can be relevant in real-life military contexts where a soldier may need to react quickly and shoot accurately.

8.5.4. Rifle disassembly and reassembly

Many occupations that require prolonged periods of wakefulness, including soldiers, also demand the ability to maintain manual dexterity. Previous research has indicated that impaired manual dexterity can be observed after 24 h of total sleep deprivation among medical professions (Banfi et al., 2019). As previously discussed, while AI has been assumed to be an effective countermeasure for preserving vigilance and attention (Malecek & Tufano, 2021), concerns have arisen about its potential adverse effects on tasks involving manual dexterity, particularly in terms of increased tearfulness or hand tremors - a typical reaction to inhalations of AI. However, in our results we did not observe any impact on rifle disassembly and reassembly time as a result of total sleep deprivation or the usage of AI. The current findings align with the results of previous studies that have been conducted on military personnel, demonstrating that a 24 h period of total sleep deprivation did not impact the manual dexterity in rifle disassembly and reassembly (Zhang et al., 2016) or the laboratory-based Grooved Pegboard test (Killgore & Kamimori, 2020).

The disparities between studies outcomes may likely be attributed to the various methods used for measuring manual dexterity in medical personnel, such as the virtual laparoscopy simulator, or to the possibility of movement automation that soldiers may develop as a result of frequent repetition of tasks during their service, referred to as a "drill." Although the present study did not specifically evaluate variables as hand steadiness, but only total time to finish a task, collectively, the evidence suggests that neither total sleep deprivation nor usage of AI has an impact on manual dexterity in military personnel.

8.5.5. Countermovement jump height and rating of perceived exertion

The presented study found that the mean countermovement jump (CMJ) height was higher among participants during the evenings as compared to all morning measurements (3.0-4.7%). The observed outcome aligns with the circadian rhythm reported in the preceding sections. Specifically, the pattern of results associated with total sleep deprivation adheres to expectations, whereas the morning session conducted after 24 h of total sleep deprivation (a point where participants were reported to be most tired based on ESS scores) does not yield worse results than those obtained after 0 h, and following 8 h of recovery sleep. Additionally, evening sessions conducted after 36 h of total sleep deprivation did not yield worse outcomes than those obtained after 12 h (Figure 10) This finding can be attributed to the increased performance in the CMJ, which demonstrates a clear circadian rhythm, with superior outcomes recorded during the afternoon relative to the morning, as described in previous research studies (Bernard et al., 1997; Thun et al., 2015). Total sleep deprivation has been suggested to affect short-term anaerobic performance, such as jumping, by reducing motivation (Blumert et al., 2007) and increased mental fatigue (Van Cutsem et al., 2017). However, this study did not collect any of such measures, leaving this interpretation of results as speculative.

Based on previous studies (Bond et al., 1986; Skein et al., 2010), total sleep deprivation may also lead to changes in the perception of effort. The RPE increased after 24 to 30 h of total sleep deprivation alongside a decrease in performance (Bond et al., 1986; Skein et al., 2010). These previous observations partly align with the results of the present study, where the highest RPE was reported after 24 h of total sleep deprivation (Figure 11A), which also corresponded with the highest level of subjective sleepiness reported by our participants (Figure 11B). Although our participants reported feeling less tired at 36 h than after 24 h (28.6%) of total sleep deprivation, this apparent "improvement" should not be interpreted as an actual improvement, but it is rather a continuation of the circadian rhythm. Eventually, the night of recovery sleep seemed to more or less returned the perception of sleepiness to levels in line with the 0 h of total sleep deprivation and normal circadian rhythm.

Our results also suggest that using AI may enhance performance in CMJ height by 1.5% and concurrently reduces RPE by 9.4%, regardless of total sleep deprivation. Notably, the observed improvements may be attributed to the psychological arousal effects of AI, such as heightened alertness, which have been documented in previous research (Campbell et al., 2022; R. R. Rogers et al., 2022). This phenomenon commonly referred to as the "psyching-up" effect of AI, may enhance short-term performance by activating the sympathetic branch of the autonomic nervous system and increasing psychological activation (Malecek & Tufano, 2021). Based on that, previous studies showed an increased peak rate of force development during the isometric mid-thigh pull exercise (Bartolomei et al., 2018; Perry et al., 2016), an explosive isometric strength task using a slow stretch-shortening cycle, the same as during CMJ. Additionally, the usage of AI increased power output over repeated high-intensity sprint exercises (R. R. Rogers et al., 2022). Regarding the following facts, while assuming a correlation between CMJ performance and short sprints (Carr et al., 2015), the usage of AI may have potential applications in some specific military scenarios, but more studies delineating optimum protocols and delivery methods are necessary.

Although previous studies have investigated the effects of total sleep deprivation and AI independently, none included the combination of these conditions. Furthermore, these studies all used various measuring techniques (CMJ with arm movement vs without; peak jump height vs average jump height; different rest periods between jumps; estimated power output vs direct assessment, etc.), all of which can impact the data, decreasing comparativeness of their findings to ours. Therefore, we acknowledge that the data from those studies bring interesting insights, but more studies with consistent methodology are still needed in order to make justifiable comparisons.

To sum up, when considering our data and the data from previous studies collectively, AI may positively impact explosive performance, regardless of the individual's sleep status. Our results also indicate that this effect may have some transfers to real-world dynamic exercises, such as jumping performance. Consequently, even though a decrease in RPE after the physical performance and improvement in short-term movements, such as jumping over a barrier or sprinting across a battlefield, may even have life-or-death consequences in military contexts, it is still necessary to take these results with caution. There is currently a shortage of published research examining the effect of AI on performance, and further research in this area is necessary to establish a more robust evidence base regarding its effect.

8.5.6. Limitations

This study's strengths comprise the tightly controlled protocol and crossover randomized controlled trial design, which allowed for direct within-subject repeated-measures investigation of the effects of total sleep deprivation and ammonia inhalation. However, study is not without limitations. For example, we were not able to perform any military-specific physically demanding tasks (e.g., casualty drag, wall climb, sprinting with personal protective equipment, loaded carries, etc.) because of limited space in the sterile laboratory-based setup. Nevertheless, the CMJ is a commonly used physical task to assess explosive neuromuscular performance, and the linear position transducer that we used is a reliable and space friendly tool for assessing CMJ. Therefore, as AI increased CMJ height, increased HR, and improved SRT in our study, future researchers should investigate the effects of AI on more demanding military-specific tasks (or tasks that last longer than the CMJ) where increasing HR or improving SRT may help aid performance.

8.6. Conclusion

Overall, despite the lack of reduction in the adverse effects of total sleep deprivation, the use of AI was found to cause a short-term increase in HR and enhance SRT without increasing the number of errors and increase CMJ height while concurrently decreasing the RPE. However, no changes were observed in handgun shooting accuracy and manual dexterity performance. These findings suggest that the utilization of AI may have potential applications in specific military scenarios, no matter the length of sleep deprivation.

9. Overall conclusion

The significance of optimal sleep in the military context cannot be understated, yet sleep deprivation remains a prevalent issue, adversely impacting performance. Therefore, the main objective of this dissertation thesis was to investigate the effects of ammonia inhalants as a fast-acting ergogenic aid, specifically assessing its ability to counteract the adverse effects of sleep deprivation and enhance performance under such challenging conditions that military personnel often operate under.

In our first research (Chapter 4), we started with collecting essential insights regarding the use and effects of ammonia inhalants on physical and cognitive performance. This review found that ammonia inhalants, while frequently used by strength-based athletes, do not substantially enhance maximal muscular strength or endurance or decrease reaction time. Notably, however, we identified limited evidence suggesting that ammonia inhalants may improve laboratory-tested explosive strength or anaerobic power performance in already fatigued athletes. The review also point to significant cardiovascular, cerebrovascular, and respiratory responses up to 30 seconds following the use of ammonia inhalants, suggesting that the timing of their use could be a critical factor in their utility as an ergogenic aid. Additionally, the review suggested that ammonia inhalants may temporarily increase beat-to-beat middle cerebral artery blood flow velocity, which has been shown to be positively associated with improved cognitive function. Our findings also discusses a potential risk factor related to ammonia inhalants use and the diagnosis of concussion, thus highlighting the need for users of ammonia inhalants athletes and their coaches to have a comprehensive understanding of ammonia inhalants use, including both potential risks and benefits. Moreover, the notion that ammonia inhalants may induce a psychological state optimal for performance warrants further examination and additional research is needed to deepen our understanding of the effects of ammonia inhalants on human physiology and cognitive performance.

Therefore, all of these understudied relationships prompted us to investigate them further in follow-up research, particularly with regard to the potential use of ammonia inhalants on military-related tasks, explicitly targeting their potential application in mitigating the adverse effects on sleep-deprived soldiers. However, before investigating the effects of ammonia inhalants on sleep-deprived soldiers, it was necessary to establish reliable testing parameters for shooting accuracy, which formed the basis of our second research. This preliminary step was essential, as it laid the foundation for evaluating the potential changes in soldiers' performance under the influence of sleep deprivation and ammonia inhalants.

Thus, our second research (Chapter 6) aimed to assess the test-retest reliability of static and dynamic shooting protocols using a laser-based handgun simulator system. Twenty soldiers participated in two trials of both shooting protocols over three separate days, with shooting accuracy analyzed using an intra-class correlation coefficient. Our results demonstrated that the tested protocols displayed good to moderate reliability between and within days. Additionally, there was no significant variation in shooting accuracy scores either between or within days, underscoring the reliability of our adopted protocols lending confidence in utilizing these static and dynamic shooting protocols in future research. This study not only contributed valuable insights into the reliable execution of shooting protocols but also, most importantly, laid a needed robust foundation for our subsequent main investigations, where we explored the effects of ammonia inhalants on military-specific physical and psychological performance in sleep-deprived soldiers.

Our last presented research (Chapter 8) investigated the potential effect of ammonia inhalants usage on simple reaction time, shooting accuracy, countermovement jump height, and rifle disassembly and reassembly time during 36 hours of total sleep deprivation, followed by 8 hours of recovery sleep on eighteen soldiers. The principal findings of our investigation were that although both total sleep deprivation and ammonia inhalants separately influenced specific parameters within the study's protocol, using ammonia inhalants did not have a sufficient effect during the sleep deprivation phase of the study. Nevertheless, regardless of sleep deprivation, results have shown that using ammonia inhalants increased heart rate for the first 15 seconds after inhalation, decreased mean simple reaction time without increasing the number of errors, and increased countermovement jump height while simultaneously decreasing the rating of perceived exertion. On the other hand, using ammonia inhalants resulted in no significant changes in shooting accuracy and the total time needed for disassembling and reassembling a rifle.

In conclusion, it is noteworthy that the observed effect of ammonia inhalants and total sleep deprivation on various performance parameters although small, may represent important in a military setting. Even though the observed differences in simple reaction time induced by ammonia inhalants were on the scale of units of milliseconds, the improvements in reaction time could be a decisive factor in military contexts where high-performance demands necessitate swift responses, potentially making the difference between life and death. In addition to that, despite ammonia inhalants not enhancing shooting accuracy, it is crucial to acknowledge that it did not lead to any degradation of any of the investigated skills. The maintained shooting accuracy and faster reaction time may prove advantageous in real-world military settings, possibly enabling soldiers to react faster in engagements.

Interestingly, lack of sleep nor the use of ammonia inhalants seemed to interfere with manual dexterity measured as the time taken to disassemble and reassemble a rifle. This may be interesting given the potential automation of movements soldiers might develop due to frequent task repetition during their service, often referred to as a "drill". Additionally, our results point towards some positive influence of ammonia inhalants' on explosive performance, independent of sleep deprivation. Increased jump height and decreased perceived exertion could prove particularly valuable in dynamic military operations, for instance, jumping over obstacles or quick sprints over the battlefield.

In sum, finding of this dissertation thesis presents the potential effects of ammonia inhalants and sleep deprivation on various aspects of performance, which we believe could have relevant for applications in military settings. However, we recognize that this investigation is merely the beginning and that further follow up and more comprehensive research is needed to fully understand these findings for improving performance in the military and other similar high-demand environments.

9.1. Conclusion of my doctoral study

During my doctoral studies, I had the privilege of cooperating with many experts in the research of strength and conditioning. The cultural and intellectual exchange of ideas with colleagues and peers from across the globe broadened my perspective and significantly enriched my knowledge and personal life. Involvement in grant projects and a team-based initiative strengthened my collaborative skills. I believe working collectively towards a common objective cultivated my ability to communicate effectively and solve problems within a diverse team structure. This insightful experience, which refined my research abilities and enhanced my interpersonal competencies, culminated in the production of several manuscripts and other written works to which I have had the opportunity to contribute. The opportunities to present our research findings at several international scientific conferences were a tremendous experience. Engaging with a global audience of researchers and academics exposed me to various perspectives and feedback that further enhanced my work.

Moreover, my internship at the Neuromuscular Research Laboratory/Warrior Human Performance Research Center at the University of Pittsburgh was an unforgettable part of my academic journey. The possibility of participating in projects focused on neuromuscular performance during sustained military operational stress expanded my understanding of the field, and the hands-on experience in this domain was invaluable in improving my practical knowledge. These experiences during my doctoral studies significantly shape my approach conduct research and guide students in their pre-graduate theses at the military department of our faculty. The expertise gained has also allowed me to better mentor and guide students in their academic plans. In effect, I feel confident I am able to further contribute to the growth and development of strength and conditioning researchers in the field of military science.

In closing, I am incredibly grateful for these opportunities, which have allowed me to grow both academically and personally. I believe this is merely the beginning of a lifelong journey of education, exploration, discovery, and contribution to the world of science.

10. References

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List of figures

presented as mean (circles) and error bars represent their 95% confidence interval (LL, UL) as an average of 15-second time spans (0-15, 15-30, 30-45, and 45-60) during shooting trials regardless of sleep deprivation (page 108).

Figure 9. Mean heart rate percentage difference (between CON and AI at each 15-second time span). Data are presented as mean (circles) and error bars represent their 95% confidence interval (LL, UL) at the average of 15-second time spans (0-15, 15-30, 30-45, and 45-60) during shooting trials regardless of sleep deprivation (page 109).

Figure 10. Mean countermovement jump height without (CON, white) and with ammonia inhalants (AI, black). Data are presented as mean (circles) and error bars represent their 95% confidence interval (LL, UL) at baseline after a full night of sleep (0 h), after 12 (-12 h), 24 (-24 h) and 36 (-36 h) hours of total sleep deprivation followed by 8 (+8 h) hours of recovery sleep (page 110).

Figure 11. (A) Mean rating of perceived exertion (RPE) without (CON, white) and with ammonia inhalants (AI, black). Data are presented as mean (circles) and error bars represent their 95% confidence interval (LL, UL) at baseline after a full night of sleep (0 h), after 12 (-12 h), 24 (-24 h) and 36 (-36 h) hours of total sleep deprivation followed by 8 (+8 h) hours of recovery sleep. **(B)** Mean Epworth Sleepiness Scale (ESS) score. Data are presented as mean (circles) and error bars represent their 95% confidence interval (LL, UL) at baseline after a full night of sleep (0 h), after 12 (-12 h), 24 (-24 h) and 36 (-36 h) hours of total sleep deprivation followed by 8 (+8 h) hours of recovery sleep (page 111).

List of tables

List of attachments

Appendix 1. Confirmation of manuscript submission and its status under review.

Appendix 2. Accepted abstract to 6th International Congress on Soldiers’ Physical Performance, London, UK, September 2023.

Appendix 3. Accepted abstract at National Strength and Conditioning Association Annual Conference, Las Vegas, NV, USA, July 2023.

Appendix 4. Presented abstract at National Strength and Conditioning Association Annual Conference, New Orleans, LA, USA, July 2022.

Appendix 5. Presented abstract at National Strength and Conditioning Association Annual Conference, New Orleans, LA, USA, July 2022

Appendix 6. Presented (online) abstract at National Strength and Conditioning Association Annual Conference, Orlando, FL, USA, July 2021.

Appendix 7. The approved form by the Ethical Committee of Faculty of Physical Education and Sport at Charles University in Prague.

Appendix 8. The approved form of informed consent by the Ethical Committee of the Faculty of Physical Education and Sport at Charles University in Prague.

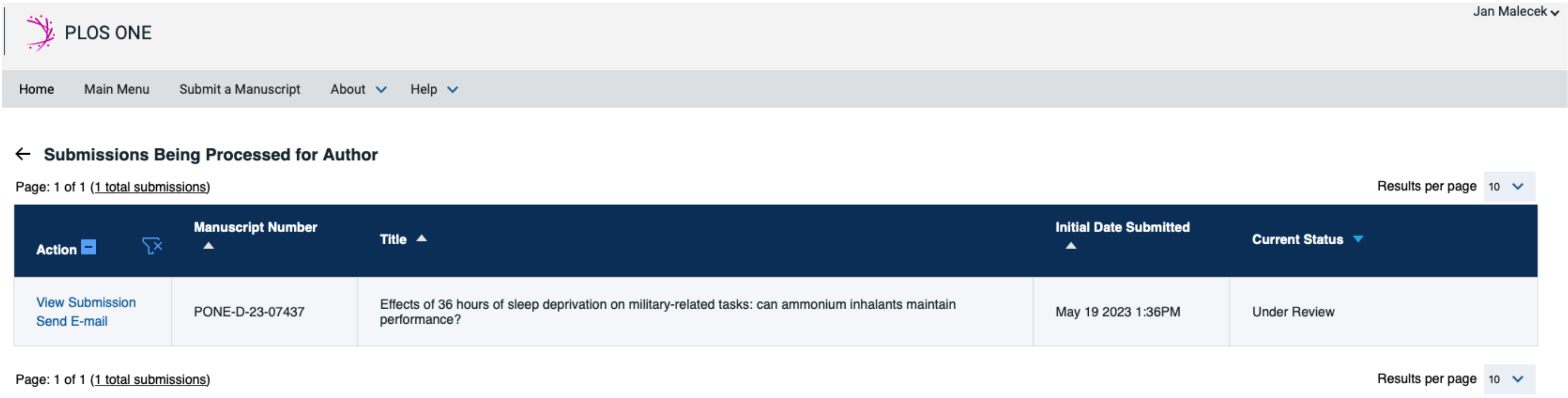
Appendix 9. The approved form by the Ethical Committee of The National Institute of Mental Health in Klecany.

Appendix 10. The approved form of informed consent by the Ethical Committee of The National Institute of Mental Health in Klecany.

Appendix 11. The Epworth Sleepiness Scale used during the research.

Appendix 12. Borg Category-Ratio 10 scale.

Appendix 1. Confirmation of manuscript submission and its status under review.



← Submissions Being Processed for Author

Page: 1 of 1 (1 total submissions)

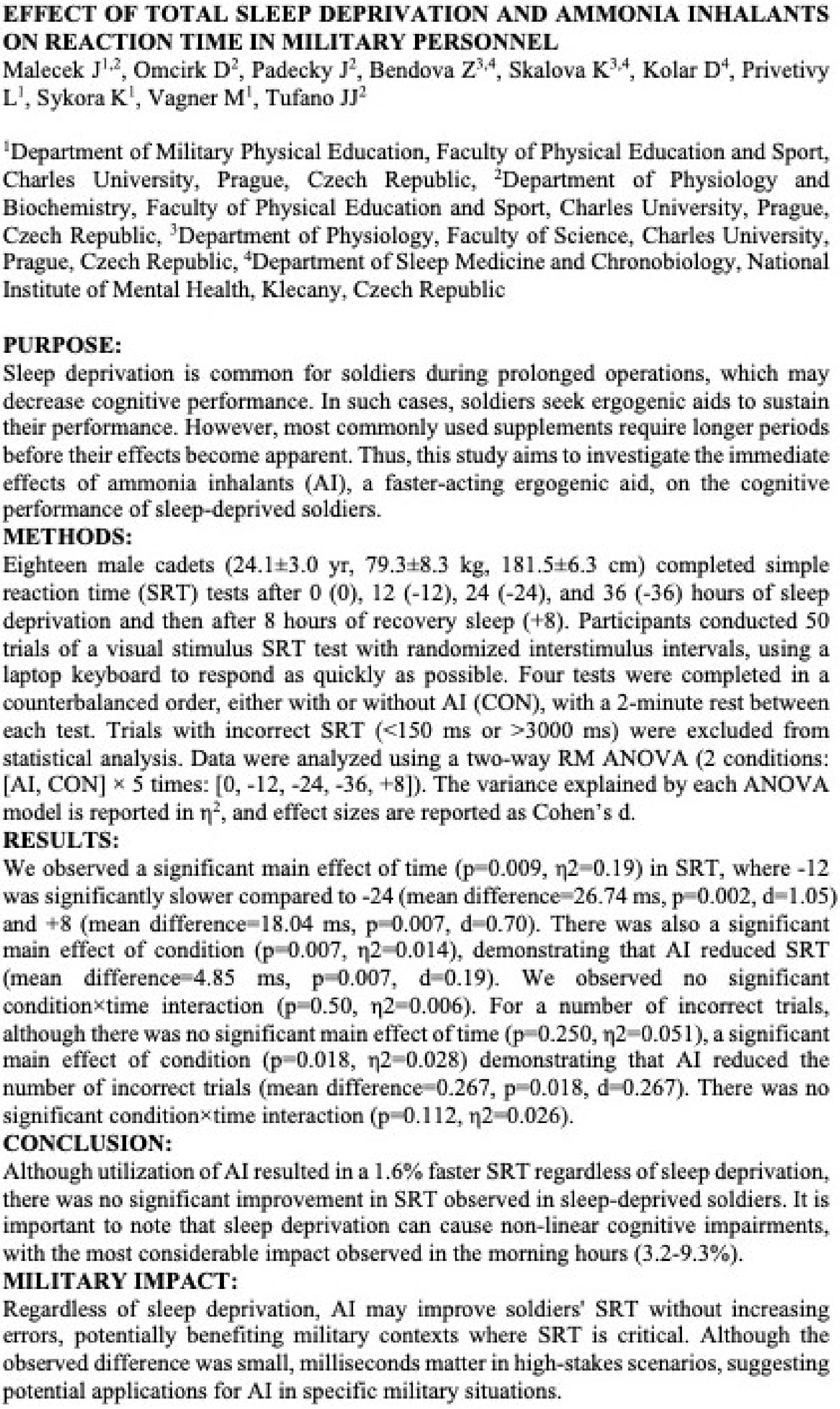
Results per page 10

Action	Manuscript Number	Title	Initial Date Submitted	Current Status
View Submission Send E-mail	PONE-D-23-07437	Effects of 36 hours of sleep deprivation on military-related tasks: can ammonium inhalants maintain performance?	May 19 2023 1:36PM	Under Review

Page: 1 of 1 (1 total submissions)

Results per page 10

Appendix 2. Accepted abstract to 6th International Congress on Soldiers' Physical Performance, London, UK, September 2023.



EFFECT OF TOTAL SLEEP DEPRIVATION AND AMMONIA INHALANTS ON REACTION TIME IN MILITARY PERSONNEL

Malecek J[1,2], Omcirk D[2], Padecky J[2], Bendova Z[3,4], Skalova K[3,4], Kolar D[4], Privetivy L[1], Sykora K[1], Vagner M[1], Tufano JJ[2]

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PURPOSE:

Sleep deprivation is common for soldiers during prolonged operations, which may decrease cognitive performance. In such cases, soldiers seek ergogenic aids to sustain their performance. However, most commonly used supplements require longer periods before their effects become apparent. Thus, this study aims to investigate the immediate effects of ammonia inhalants (AI), a faster-acting ergogenic aid, on the cognitive performance of sleep-deprived soldiers.

METHODS:

Eighteen male cadets (24.1±3.0 yr, 79.3±8.3 kg, 181.5±6.3 cm) completed simple reaction time (SRT) tests after 0 (0), 12 (-12), 24 (-24), and 36 (-36) hours of sleep deprivation and then after 8 hours of recovery sleep (+8). Participants conducted 50 trials of a visual stimulus SRT test with randomized interstimulus intervals, using a laptop keyboard to respond as quickly as possible. Four tests were completed in a counterbalanced order, either with or without AI (CON), with a 2-minute rest between each test. Trials with incorrect SRT (<150 ms or >3000 ms) were excluded from statistical analysis. Data were analyzed using a two-way RM ANOVA (2 conditions: [AI, CON] × 5 times: [0, -12, -24, -36, +8]). The variance explained by each ANOVA model is reported in η^2, and effect sizes are reported as Cohen's d.

RESULTS:

We observed a significant main effect of time (p=0.009, η2=0.19) in SRT, where -12 was significantly slower compared to -24 (mean difference=26.74 ms, p=0.002, d=1.05) and +8 (mean difference=18.04 ms, p=0.007, d=0.70). There was also a significant main effect of condition (p=0.007, η2=0.014), demonstrating that AI reduced SRT (mean difference=4.85 ms, p=0.007, d=0.19). We observed no significant condition×time interaction (p=0.50, η2=0.006). For a number of incorrect trials, although there was no significant main effect of time (p=0.250, η2=0.051), a significant main effect of condition (p=0.018, η2=0.028) demonstrating that AI reduced the number of incorrect trials (mean difference=0.267, p=0.018, d=0.267). There was no significant condition×time interaction (p=0.112, η2=0.026).

CONCLUSION:

Although utilization of AI resulted in a 1.6% faster SRT regardless of sleep deprivation, there was no significant improvement in SRT observed in sleep-deprived soldiers. It is important to note that sleep deprivation can cause non-linear cognitive impairments, with the most considerable impact observed in the morning hours (3.2-9.3%).

MILITARY IMPACT:

Regardless of sleep deprivation, AI may improve soldiers' SRT without increasing errors, potentially benefiting military contexts where SRT is critical. Although the observed difference was small, milliseconds matter in high-stakes scenarios, suggesting potential applications for AI in specific military situations.

Appendix 3. Accepted abstract at National Strength and Conditioning Association Annual Conference, Las Vegas, NV, USA, July 2023.

EFFECT OF TOTAL SLEEP DEPRIVATION AND AMMONIA INHALANTS ON RATING OF PERCEIVED EXERTION IN MILITARY PERSONNEL

Malecek J[1] • Omcirk D[1] • Padecky J[1] • Bendova Z[2] • Skalova K[2] • Kolar D[2] • Privetivy L[1] • Sykora K[1] • Vagner M[1] • Tufano JJ[1]

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[2]National Institute of Mental Health, Klecany, Czech Republic

PURPOSE

Previous literature suggests that sleep deprivation is associated with an increased rating of perceived exertion (RPE). Given that soldiers may be required to function under conditions of sleep deprivation for extended periods, lasting up to 24 hr or more, they may resort to fast-acting ergogenic aids, such as ammonia inhalants (AI). Therefore, this study aimed to determine the effects of AI on the RPE of sleep-deprived soldiers.

METHODS

Eighteen male cadets (24.1 ± 3.0 yr, 79.3 ± 8.3 kg, 181.5 ± 6.3 cm) were exposed to 36-hr of total sleep deprivation during a crossover randomized controlled trial using a within-subjects repeated-measure design. They performed countermovement jump (CMJ) testing in the morning (0), after 12-hr (-12), 24-hr (-24), and 36-hr of total sleep deprivation (-36) in addition to another testing after 8-hr of recovery sleep (+8). Each CMJ test included 2 sets of 3 maximal effort CMJs, each set was performed either with AI (AI) or without (CON), in a counterbalanced order) with 2 min inter-set rest. All CMJs were performed with wooden dowel (0.5 kg) as a mock barbell placed across the participant's upper back, mimicking a regular back squat (CMJ depth was self-selected). The researcher verbally instructed and encouraged the participants to jump as high as possible on each jump. Following each set of CMJs, the RPE for each condition at each time point was recorded using a CR-10 scale. Data were analyzed using a two-way RM ANOVA (2 conditions: [AI, CON] × 5 times: [0, -12, -24, -36, +8]) to access the differences in RPE. The variance explained by each ANOVA model is reported in η2, and the mean difference effect sizes are reported as Cohen's d with 95% confidence intervals [LL, UL].

RESULTS

We observed a statistically significant main effect of time (F2.844, 10.030 = 10.478, $p < 0.001$, η2 = 0.282), indicating that soldiers reported the highest RPE at -24 compared to 0 (mean difference = 1.11, $p < 0.001$, d = 1.19 [0.32, 2.06]), -12 (mean difference = 1.03, p = 0.010, d = 1.10 [-0.26, 1.93]), and +8 (mean difference = 0.86, p = 0.002, d = 0.92 [0.14, 1.70]). There was also a statistically significant main effect of condition (F1, 3.472 = 17.220, $p < 0.001$, η2 = 0.034), demonstrating that soldiers reported higher RPE at CON compared to AI (mean difference = 0.28, $p < 0.001$, d = 0.30 [0.11, 0.48]). There was no statistically significant condition × time interaction (F4, 0.264 = 0.968, p = 0.431, η2 = 0.010).

CONCLUSION

There was no significant interaction of using AI regarding decreased RPE in sleep-deprived soldiers. However, we observed a reduction in RPE (0.28, 9.4%) for CMJs performed with AI, regardless of the length of sleep deprivation. A possible explanation may be related to the psychological arousal effects often reported by strength-based athletes, accompanied by increased levels of consciousness and alertness in response to AI. This phenomenon, commonly called the "psyching-up" effect after AI usage, is caused by activating the sympathetic branch of the autonomic nervous system and increasing psychological activation. Based on our results, it could lead to a lowering of the rating of activity demands perception in soldiers.

CONTACT AND ACKNOWLEDGEMENTS

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This study was partly funded by GAUK 986120.

PRACTICAL APPLICATIONS

Tactical strength and conditioning professionals may consider having AI available for the soldiers since AI seem to decrease RPE during short-term explosive lower body power performance. Thus, it may have potential applications in specific military scenarios.

Fig 1. Illustration of the ammonia inhalants administration process, showcasing the immediate positioning of the capsule under the participant's nose and inhaling until a voluntary withdrawal reflex is induced.

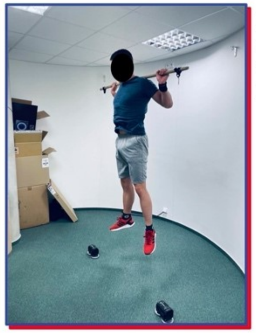

Fig 2. Subject performing a countermovement jump testing.

Appendix 4. Presented abstract at National Strength and Conditioning Association Annual Conference, New Orleans, LA, USA, July 2022.

EFFECT OF TOTAL SLEEP DEPRIVATION AND AMMONIA INHALANTS ON COUNTERMOVEMENT JUMP PERFORMANCE IN MILITARY PERSONNEL

Malecek J[1] • Omcirk D[1] • Padecky J[1] • Bendova Z[2] • Skalova K[2] • Kolar D[2] • Privetivy L[1] • Sykora K[1] • Vagner M[1] • Tufano JJ[1]

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PURPOSE

Although soldiers may experience periods of sleep deprivation, adequate sleep is needed to maintain optimal health and tactical performance in military service members. As sleep deprivation may decrease physical performance, soldiers may look to various ergogenic aids to help sustain performance. As many common supplements require lengthy periods of time before their effects can be seen (e.g., caffeine), a faster-acting ergogenic aid (e.g., ammonia inhalants [AIs]) could be useful. Therefore, the purpose of this study was to determine the effects of AIs on the physical performance of sleep-deprived soldiers.

METHODS

Eighteen active-duty men from the Czech military (24.1 ± 3.0 y, 79.3 ± 8.1 kg, 181.5 ± 6.1 cm) were exposed to ~36 hr of total sleep deprivation during a crossover randomized controlled trial using a within-subjects repeated-measures design. They performed countermovement jump (CMJ) testing (Fig. 2) in the morning (0), after 12-hr (-12), 24-hr (-24), and 36-hr of total sleep deprivation (-36) in addition to another CMJ test after 8-hr of recovery sleep (+8). Each CMJ test included 2 sets of 3 maximal effort CMJs (each set was performed either with or without AIs, in a counterbalanced order) with 2 min inter-set rest. All CMJs were performed with a wooden dowel across the upper back with a linear position transducer attached from which the mean of the 3 peak jump heights (JH) was assessed for each AIs condition at each time point. A two-way 2 (condition: with AIs, without AIs) x 5 (time: 0, -12, -24, -36, +8) repeated-measures ANOVA ($p \leq 0.05$) was conducted to determine differences in JH.

RESULTS

There was no condition x time interaction. However, there was a main effect of condition where JH was greater with AIs than without (Fig. 1). There was also the main effect of time where JH was always lower in the morning (0, -24, and +8) than the evening (-12 and -36) regardless of the conditions (Fig. 1).

CONCLUSION

There was no significant interaction of using AIs in terms of increased JH in sleep-deprived soldiers, but AIs did increase JH regardless of sleep deprivation. Moreover, soldiers jumped higher in the evenings than in the mornings regardless of sleep deprivation or AIs usage.

PRACTICAL APPLICATIONS

Based on these data, tactical strength and conditioning professionals may consider having AIs available for the soldiers, regardless of sleep deprivation, since AIs seem to enhance short-term explosive lower body power performance. Additionally, future research should consider some longer-lasting performance since the effect of AIs may last only ~30 s.

CONTACT

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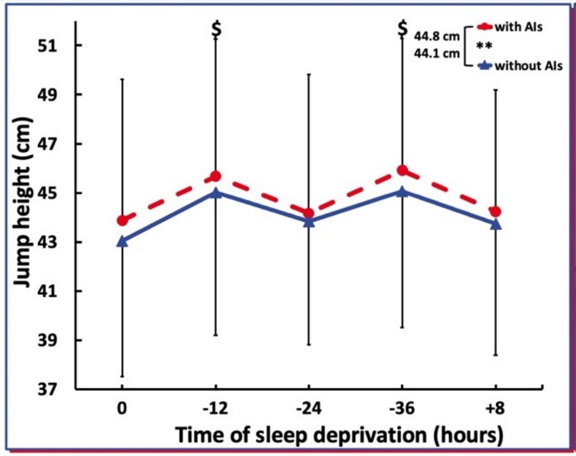

Fig 1. Countermovement jump height **without** and **with** ammonia inhalants (AIs). Data are presented as mean ± standard deviation at a morning baseline (0), and after 12 (-12), 24 (-24), and 36 (-36) hours of sleep deprivation followed by 8 hours of sleep (+8).
****** indicates a significant main effect of the condition.
$ indicates a significant main effect of time (greater than 0, -24, and +8).

Fig 2. Subject performing countermovement jump testing.

ACKNOWLEDGEMENTS

This study was partly funded by GAUK 986120.

Appendix 5. Presented abstract at National Strength and Conditioning Association Annual Conference, New Orleans, LA, USA, July 2022

EFFECT OF TOTAL SLEEP DEPRIVATION AND AMMONIA INHALANTS ON HANDGUN SHOOTING ACCURACY IN MILITARY PERSONNEL

Malecek J[1] • Omcirk D[1] • Padecky J[1] • Bendova Z[2] • Skalova K[2] • Kolar D[2] • Privetivy L[1] • Sykora K[1] • Vagner M[1] • Tufano JJ[1]

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PURPOSE

Operational demands can cause soldiers to operate under total sleep deprivation for up to 24 hours or more. Although reduced sleep can impair long-distance shooting accuracy with a military rifle, the effect of reduced sleep on close-range handgun shooting accuracy remains unclear. Additionally, to mitigate the adverse effects of sleep deprivation on shooting accuracy, soldiers may look to fast-acting ergogenic aids (e.g., ammonia inhalants [AIs]). Therefore, this study investigated the effects of AIs on handgun shooting accuracy in sleep-deprived soldiers.

METHODS

Eighteen active-duty men from the Czech military (24.1 ± 3.0 y, 79.3 ± 8.1 kg, 181.5 ± 6.1 cm) were exposed to ~36-hr of total sleep deprivation during a crossover randomized controlled trial using a within-subjects repeated-measures design. They performed a reliable (ICC: 0.869) static shooting protocol in the morning (0), after 12-hr (-12), 24-hr (-24), and 36-hr of total sleep deprivation (-36), and after 8-hr of recovery sleep (+8). The shooting protocol was performed with a laser-based simulator system (LASRX) with a real-weight military laser pistol (GLOCK 17/22) and was performed twice in a standing position with a 1-min time limit and 2-min of inter-protocol rest (either with or without AIs, in a counterbalanced order). For all shots, a 20 cm circular target was positioned 4 m in front of the soldier to simulate a standard-issue 50 cm target at 10 m. During the protocol, soldiers fired 10 shots, aiming to hit the middle of the circular target (a bullseye was worth 10 points, and 1 point was deducted for every 2 cm region from the bullseye, resulting in a maximum score of 100 points). Soldiers wore over-ear headphones to hear the command to start shooting and the simulated shooting blasts when pulling the trigger. A two-way 2 (condition: with AIs, without AIs) x 5 (time: 0, -12, -24, -36, +8) repeated-measures ANOVA ($p \leq 0.05$) was conducted to determine differences in shooting accuracy at each time point both with and without AIs.

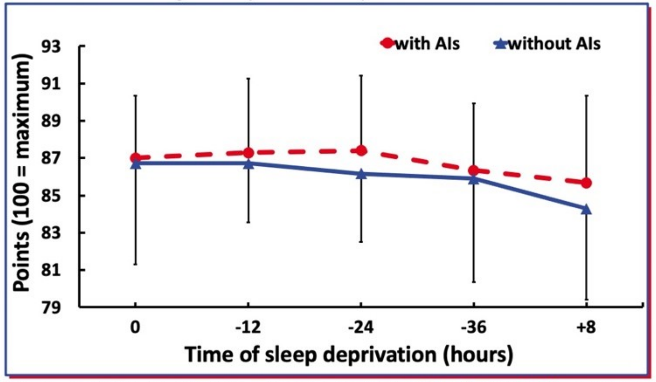

Fig 1. Accuracy of handgun shooting (points) without and with ammonia inhalants (AIs). Data are presented as mean ± standard deviation at a morning baseline (0), and after 12 (-12), 24 (-24), and 36 (-36) hours of sleep deprivation followed by 8 hours of recovery sleep (+8).

ACKNOWLEDGEMENTS

This study was partly funded by GAUK 986120.

Fig 2. Subject performing a shooting protocol.

Fig 3. Subject inhaling AIs before a shooting protocol.

RESULTS

There was no condition*time interaction, main effect of time, or a main effect of condition (Fig. 1).

CONCLUSION

Although previous research has shown that sleep deprivation may decrease long-distance rifle shooting accuracy, there was no effect of AIs or sleep deprivation on close-range handgun accuracy in soldiers.

PRACTICAL APPLICATIONS

Fast-acting ergogenic aids do not improve handgun shooting accuracy, even in the presence of sleep deprivation. Therefore, other ergogenic aids are worth investigating in this context.

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Appendix 6. Presented (online) abstract at National Strength and Conditioning Association Annual Conference, Orlando, FL, USA, July 2021.

RELIABILITY OF A MARKSMANSHIP TEST WHILE USING A LASER-BASED MARKSMANSHIP SIMULATOR SYSTEM IN MILITARY PERSONNEL

Malecek J • Omcirk D • Didek Z • Vetrovsky T • Tufano JJ

Faculty of Physical Education and Sport, Charles University, Prague, Czech Republic

PURPOSE

Tactical strength and conditioning facilitators may seek to determine the effects of neuromuscular fatigue on shooting accuracy, but data regarding the reliability of standard static and dynamic lab-based shooting tests is lacking. The purpose of this research was to determine the reliability of military standard-issued static and dynamic marksmanship tests executed by a laser-based marksmanship simulator system.

METHODS

Twenty soldiers (22 ± 1.9 y; 81.7 ± 8.6 kg; 184.9 ± 5.9 cm) performed a familiarization visit, and three subsequent experimental visits separated by 24 hours. Each visit included a static and dynamic laser-based marksmanship protocol performed in a standing position. For all shots, a 20 cm circular target was on a blank wall 4 meters in front of the participant to simulate a standard-issue 50 cm target at 10 meters. During each experimental visit, subjects practiced both protocols and then performed the static protocol where they fired ten shots, aiming to hit the middle of the circular target. Following 1 minute of rest, the dynamic protocol was performed where they fired three shots in a row at three targets from left-to-right (1-meter apart), for a total of nine shots. Each protocol was performed twice with a 1-minute time limit and 1-minute of inter-protocol rest. The best possible score was 100 (static) and 90 (dynamic) points respectively. The sum of each protocol was recorded for future analysis. A laser-based marksmanship simulator system (LASRX) and real-weight laser military assault pistol mockup (GLOCK 17/22) were used. Subjects wore over-ear headphones to hear the command to start shooting and the simulated pistol shooting blasts when pulling the trigger. Lin's concordance correlation coefficient (CCC) was used to assess the test-retest reliability of the marksmanship test by measuring the variation from the 45° line through the origin (the concordance line). The CCC is analogous to the intraclass correlation coefficient (ICC) in that it takes values between -1 and 1, where -1 means perfect disagreement, 0 signifies complete independence, and 1 indicates perfect agreement. The CCC's advantage is that, unlike the ICC, it considers both systematic bias and random error when assessing agreement between a test and a retest. The CCCs and their 95% confidence intervals were calculated separately for the static and dynamic protocol and for within- and between-day reliability.

RESULTS

For the static protocol, the CCC was 0.569 (95% CI: 0.357 to 0.725) for within-day test-retest reliability and 0.719 (0.597 to 0.808) for between day reliability. For the dynamic protocol, the CCC was 0.516 (0.300 to 0.682) for within-day reliability and 0.690 (0.517 to 0.809) for between-day reliability.

CONCLUSION

Although the absence of an initial recoil during laser-based marksmanship may not be identical to real shooting, the laboratory-based laser tests were moderately reliable for determining shooting accuracy. However, future research should investigate the comparison of simulated and real-gun marksmanship tasks to shed light on whether and how much low-cost laser-based marksmanship corresponds to reality.

PRACTICAL APPLICATIONS

The laser-based marksmanship simulator systems can serve as a moderately reliable approach for assessing shooting accuracy, thus providing a way to assess the effects of neuromuscular fatigue on a military-specific shooting task.

Figure 1. The 20 cm circular target (modified from a standard-issue 50 cm).

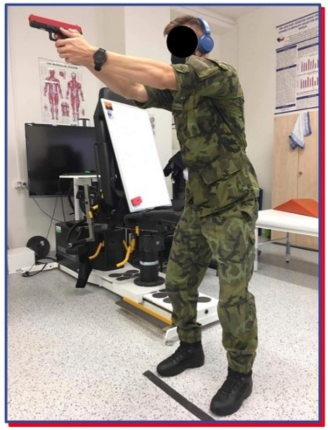

Figure 2. A participant is performing laser-based marksmanship protocol.

ACKNOWLEDGEMENTS

This study was partly funded by GAUK 986120.

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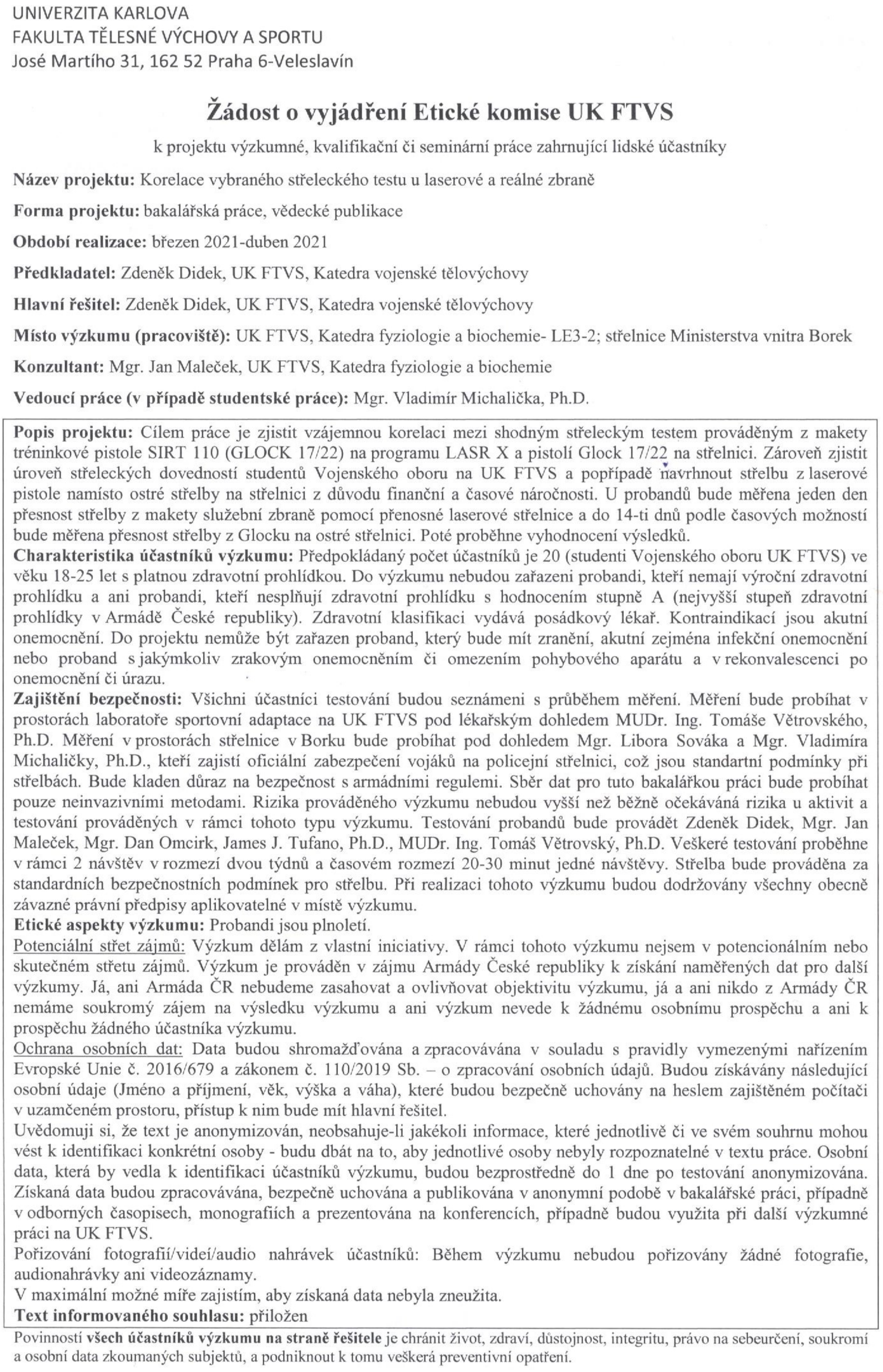
Appendix 7. The approved form by the Ethical Committee of Faculty of Physical Education and Sport at Charles University in Prague.



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Žádost o vyjádření Etické komise UK FTVS

k projektu výzkumné, kvalifikační či seminární práce zahrnující lidské účastníky

Název projektu: Korelace vybraného střeleckého testu u laserové a reálné zbraně

Forma projektu: bakalářská práce, vědecké publikace

Období realizace: březen 2021-duben 2021

Předkladatel: Zdeněk Didek, UK FTVS, Katedra vojenské tělovýchovy

Hlavní řešitel: Zdeněk Didek, UK FTVS, Katedra vojenské tělovýchovy

Místo výzkumu (pracoviště): UK FTVS, Katedra fyziologie a biochemie- LE3-2; střelnice Ministerstva vnitra Borek

Konzultant: Mgr. Jan Maleček, UK FTVS, Katedra fyziologie a biochemie

Vedoucí práce (v případě studentské práce): Mgr. Vladimír Michalička, Ph.D.

Popis projektu: Cílem práce je zjistit vzájemnou korelaci mezi shodným střeleckým testem prováděným z makety tréninkové pistole SIRT 110 (GLOCK 17/22) na programu LASR X a pistolí Glock 17/22 na střelnici. Zároveň zjistit úroveň střeleckých dovedností studentů Vojenského oboru na UK FTVS a popřípadě navrhnout střelbu z laserové pistole namísto ostré střelby na střelnici z důvodu finanční a časové náročnosti. U probandů bude měřena jeden den přesnost střelby z makety služební zbraně pomocí přenosné laserové střelnice a do 14-ti dnů podle časových možností bude měřena přesnost střelby z Glocku na ostré střelnici. Poté proběhne vyhodnocení výsledků.
Charakteristika účastníků výzkumu: Předpokládaný počet účastníků je 20 (studenti Vojenského oboru UK FTVS) ve věku 18-25 let s platnou zdravotní prohlídkou. Do výzkumu nebudou zařazeni probandi, kteří nemají výroční zdravotní prohlídku a ani probandi, kteří nesplňují zdravotní prohlídku s hodnocením stupně A (nejvyšší stupeň zdravotní prohlídky v Armádě České republiky). Zdravotní klasifikaci vydává posádkový lékař. Kontraindikací jsou akutní onemocnění. Do projektu nemůže být zařazen proband, který bude mít zranění, akutní zejména infekční onemocnění nebo proband s jakýmkoliv zrakovým onemocněním či omezením pohybového aparátu a v rekonvalescenci po onemocnění či úrazu.
Zajištění bezpečnosti: Všichni účastníci testování budou seznámeni s průběhem měření. Měření bude probíhat v prostorách laboratoře sportovní adaptace na UK FTVS pod lékařským dohledem MUDr. Ing. Tomáše Větrovského, Ph.D. Měření v prostorách střelnice v Borku bude probíhat pod dohledem Mgr. Libora Sováka a Mgr. Vladimíra Michaličky, Ph.D., kteří zajistí oficiální zabezpečení vojáků na policejní střelnici, což jsou standartní podmínky při střelbách. Bude kladen důraz na bezpečnost s armádními regulemi. Sběr dat pro tuto bakalářkou práci bude probíhat pouze neinvazivními metodami. Rizika prováděného výzkumu nebudou vyšší než běžně očekáváná rizika u aktivit a testování prováděných v rámci tohoto typu výzkumu. Testování probandů bude provádět Zdeněk Didek, Mgr. Jan Maleček, Mgr. Dan Omcirk, James J. Tufano, Ph.D., MUDr. Ing. Tomáš Větrovský, Ph.D. Veškeré testování proběhne v rámci 2 návštěv v rozmezí dvou týdnů a časovém rozmezí 20-30 minut jedné návštěvy. Střelba bude prováděna za standardních bezpečnostních podmínek pro střelbu. Při realizaci tohoto výzkumu budou dodržovány všechny obecně závazné právní předpisy aplikovatelné v místě výzkumu.
Etické aspekty výzkumu: Probandi jsou plnoletí.
Potenciální střet zájmů: Výzkum dělám z vlastní iniciativy. V rámci tohoto výzkumu nejsem v potencionálním nebo skutečném střetu zájmů. Výzkum je prováděn v zájmu Armády České republiky k získání naměřených dat pro další výzkumy. Já, ani Armáda ČR nebudeme zasahovat a ovlivňovat objektivitu výzkumu, já a ani nikdo z Armády ČR nemáme soukromý zájem na výsledku výzkumu a ani výzkum nevede k žádnému osobnímu prospěchu a ani k prospěchu žádného účastníka výzkumu.
Ochrana osobních dat: Data budou shromažďována a zpracovávána v souladu s pravidly vymezenými nařízením Evropské Unie č. 2016/679 a zákonem č. 110/2019 Sb. – o zpracování osobních údajů. Budou získávány následující osobní údaje (Jméno a příjmení, věk, výška a váha), které budou bezpečně uchovány na heslem zajištěném počítači v uzamčeném prostoru, přístup k nim bude mít hlavní řešitel.
Uvědomuji si, že text je anonymizován, neobsahuje-li jakékoli informace, které jednotlivě či ve svém souhrnu mohou vést k identifikaci konkrétní osoby - budu dbát na to, aby jednotlivé osoby nebyly rozpoznatelné v textu práce. Osobní data, která by vedla k identifikaci účastníků výzkumu, budou bezprostředně do 1 dne po testování anonymizována. Získaná data budou zpracovávána, bezpečně uchována a publikována v anonymní podobě v bakalářské práci, případně v odborných časopisech, monografiích a prezentována na konferencích, případně budou využita při další výzkumné práci na UK FTVS.
Pořizování fotografií/videí/audio nahrávek účastníků: Během výzkumu nebudou pořizovány žádné fotografie, audionahrávky ani videozáznamy.
V maximální možné míře zajistím, aby získaná data nebyla zneužita.
Text informovaného souhlasu: přiložen

Povinností **všech účastníků výzkumu na straně řešitele** je chránit život, zdraví, důstojnost, integritu, právo na sebeurčení, soukromí a osobní data zkoumaných subjektů, a podniknout k tomu veškerá preventivní opatření.

UNIVERZITA KARLOVA
FAKULTA TĚLESNÉ VÝCHOVY A SPORTU
José Martího 31, 162 52 Praha 6-Veleslavín

Odpovědnost za ochranu zkoumaných subjektů leží vždy na účastnících výzkumu na straně řešitele, nikdy na zkoumaných, byť dali svůj souhlas k účasti na výzkumu. Všichni účastníci výzkumu na straně řešitele musí brát v potaz etické, právní a regulační normy a standardy výzkumu na lidských subjektech, které platí v České republice, stejně jako ty, jež platí mezinárodně.
Potvrzuji, že tento popis projektu odpovídá návrhu realizace projektu a že při jakékoli změně projektu, zejména použitých metod, zašlu Etické komisi UK FTVS revidovanou žádost.

V Praze dne: 8.1.2021

Podpis předkladatele:

Datum a podpis odpovědného pracovníka z místa výzkumu:

Vyjádření Etické komise UK FTVS

Složení komise: **Předsedkyně:** doc. PhDr. Irena Parry Martínková, Ph.D.
Členové: prof. MUDr. Jan Heller, CSc.
prof. PhDr. Pavel Slepička, DrSc.
PhDr. Pavel Hráský, Ph.D.
Mgr. Eva Prokešová, Ph.D.
Mgr. Tomáš Ruda, Ph.D.
MUDr. Simona Majorová

Projekt práce byl schválen Etickou komisí UK FTVS pod jednacím číslem: 224/2020
dne: 8. 3. 2021

Etická komise UK FTVS zhodnotila předložený projekt a **neshledala žádné rozpory** s platnými zásadami, předpisy a mezinárodní směrnicemi pro provádění výzkumu zahrnujícího lidské účastníky.

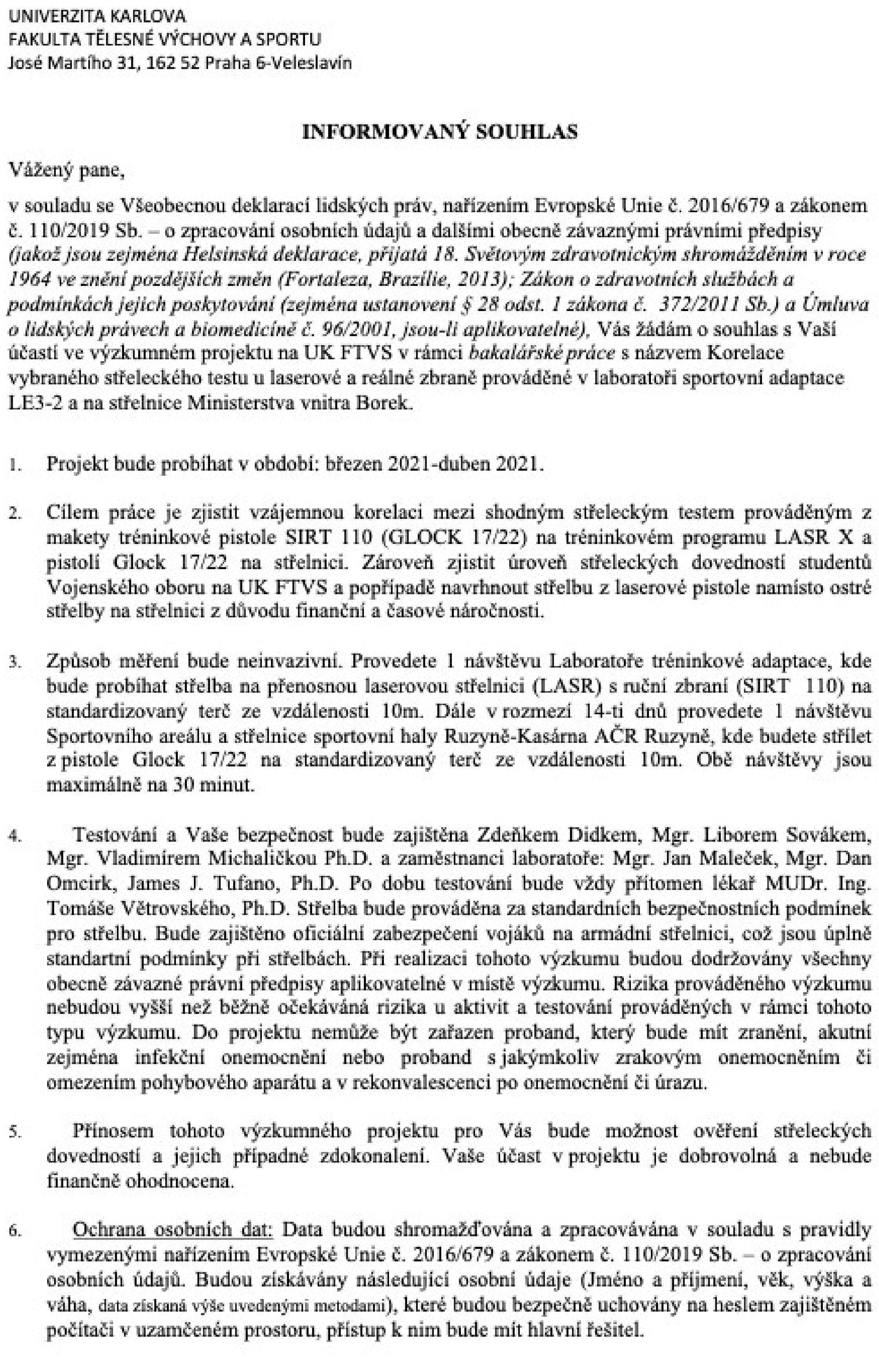
Řešitel projektu splnil podmínky nutné k získání souhlasu Etické komise.

UNIVERZITA KARLOVA
Fakulta tělesné výchovy a sportu
José Martího 31, 162 52, Praha 6
– 20 –

razítko UK FTVS

podpis předsedkyně EK UK FTVS

Appendix 8. The approved form of informed consent by the Ethical Committee of the Faculty of Physical Education and Sport at Charles University in Prague.



UNIVERZITA KARLOVA
FAKULTA TĚLESNÉ VÝCHOVY A SPORTU
José Martího 31, 162 52 Praha 6-Veleslavín

INFORMOVANÝ SOUHLAS

Vážený pane,

v souladu se Všeobecnou deklarací lidských práv, nařízením Evropské Unie č. 2016/679 a zákonem č. 110/2019 Sb. – o zpracování osobních údajů a dalšími obecně závaznými právními předpisy *(jakož jsou zejména Helsinská deklarace, přijatá 18. Světovým zdravotnickým shromážděním v roce 1964 ve znění pozdějších změn (Fortaleza, Brazílie, 2013); Zákon o zdravotních službách a podmínkách jejich poskytování (zejména ustanovení § 28 odst. 1 zákona č. 372/2011 Sb.) a Úmluva o lidských právech a biomedicíně č. 96/2001, jsou-li aplikovatelné),* Vás žádám o souhlas s Vaší účastí ve výzkumném projektu na UK FTVS v rámci *bakalářské práce* s názvem Korelace vybraného střeleckého testu u laserové a reálné zbraně prováděné v laboratoři sportovní adaptace LE3-2 a na střelnice Ministerstva vnitra Borek.

1. Projekt bude probíhat v období: březen 2021-duben 2021.

2. Cílem práce je zjistit vzájemnou korelaci mezi shodným střeleckým testem prováděným z makety tréninkové pistole SIRT 110 (GLOCK 17/22) na tréninkovém programu LASR X a pistolí Glock 17/22 na střelnici. Zároveň zjistit úroveň střeleckých dovedností studentů Vojenského oboru na UK FTVS a popřípadě navrhnout střelbu z laserové pistole namísto ostré střelby na střelnici z důvodu finanční a časové náročnosti.

3. Způsob měření bude neinvazivní. Provedete 1 návštěvu Laboratoře tréninkové adaptace, kde bude probíhat střelba na přenosnou laserovou střelnici (LASR) s ruční zbraní (SIRT 110) na standardizovaný terč ze vzdálenosti 10m. Dále v rozmezí 14-ti dnů provedete 1 návštěvu Sportovního areálu a střelnice sportovní haly Ruzyně-Kasárna AČR Ruzyně, kde budete střílet z pistole Glock 17/22 na standardizovaný terč ze vzdálenosti 10m. Obě návštěvy jsou maximálně na 30 minut.

4. Testování a Vaše bezpečnost bude zajištěna Zdeňkem Didkem, Mgr. Liborem Sovákem, Mgr. Vladimírem Michaličkou Ph.D. a zaměstnanci laboratoře: Mgr. Jan Maleček, Mgr. Dan Omcirk, James J. Tufano, Ph.D. Po dobu testování bude vždy přítomen lékař MUDr. Ing. Tomáše Větrovského, Ph.D. Střelba bude prováděna za standardních bezpečnostních podmínek pro střelbu. Bude zajištěno oficiální zabezpečení vojáků na armádní střelnici, což jsou úplně standartní podmínky při střelbách. Při realizaci tohoto výzkumu budou dodržovány všechny obecně závazné právní předpisy aplikovatelné v místě výzkumu. Rizika prováděného výzkumu nebudou vyšší než běžně očekávána rizika u aktivit a testování prováděných v rámci tohoto typu výzkumu. Do projektu nemůže být zařazen proband, který bude mít zranění, akutní zejména infekční onemocnění nebo proband s jakýmkoliv zrakovým onemocněním či omezením pohybového aparátu a v rekonvalescenci po onemocnění či úrazu.

5. Přínosem tohoto výzkumného projektu pro Vás bude možnost ověření střeleckých dovedností a jejich případné zdokonalení. Vaše účast v projektu je dobrovolná a nebude finančně ohodnocena.

6. Ochrana osobních dat: Data budou shromažďována a zpracovávána v souladu s pravidly vymezenými nařízením Evropské Unie č. 2016/679 a zákonem č. 110/2019 Sb. – o zpracování osobních údajů. Budou získávány následující osobní údaje (Jméno a příjmení, věk, výška a váha, data získaná výše uvedenými metodami), které budou bezpečně uchovány na heslem zajištěném počítači v uzamčeném prostoru, přístup k nim bude mít hlavní řešitel.

Uvědomuji si, že text je anonymizován, neobsahuje-li jakékoli informace, které jednotlivě či ve svém souhrnu mohou vést k identifikaci konkrétní osoby – budu dbát na to, aby jednotlivé osoby nebyly rozpoznatelné v textu práce.
Osobní data, která by vedla k identifikaci účastníků výzkumu, budou bezprostředně do 1 dne po testování anonymizována. Získaná data budou zpracovávána, bezpečně uchována a publikována v anonymní podobě v bakalářské práci, případně v odborných časopisech, monografiích a prezentována na konferencích, případně budou využita při další výzkumné práci na UK FTVS.
Pořizování fotografií/videí/audio nahrávek účastníků: Během výzkumu nebudou pořizovány žádné fotografie, audionahrávky ani videozáznamy.
V maximální možné míře zajistím, aby získaná data nebyla zneužita.

7. S celkovými výsledky a závěry výzkumného projektu se můžete seznámit na e-mailové adrese zdenek.didek@gmail.com

Jméno a příjmení předkladatele a hlavního řešitele projektu: Zdeněk Didek

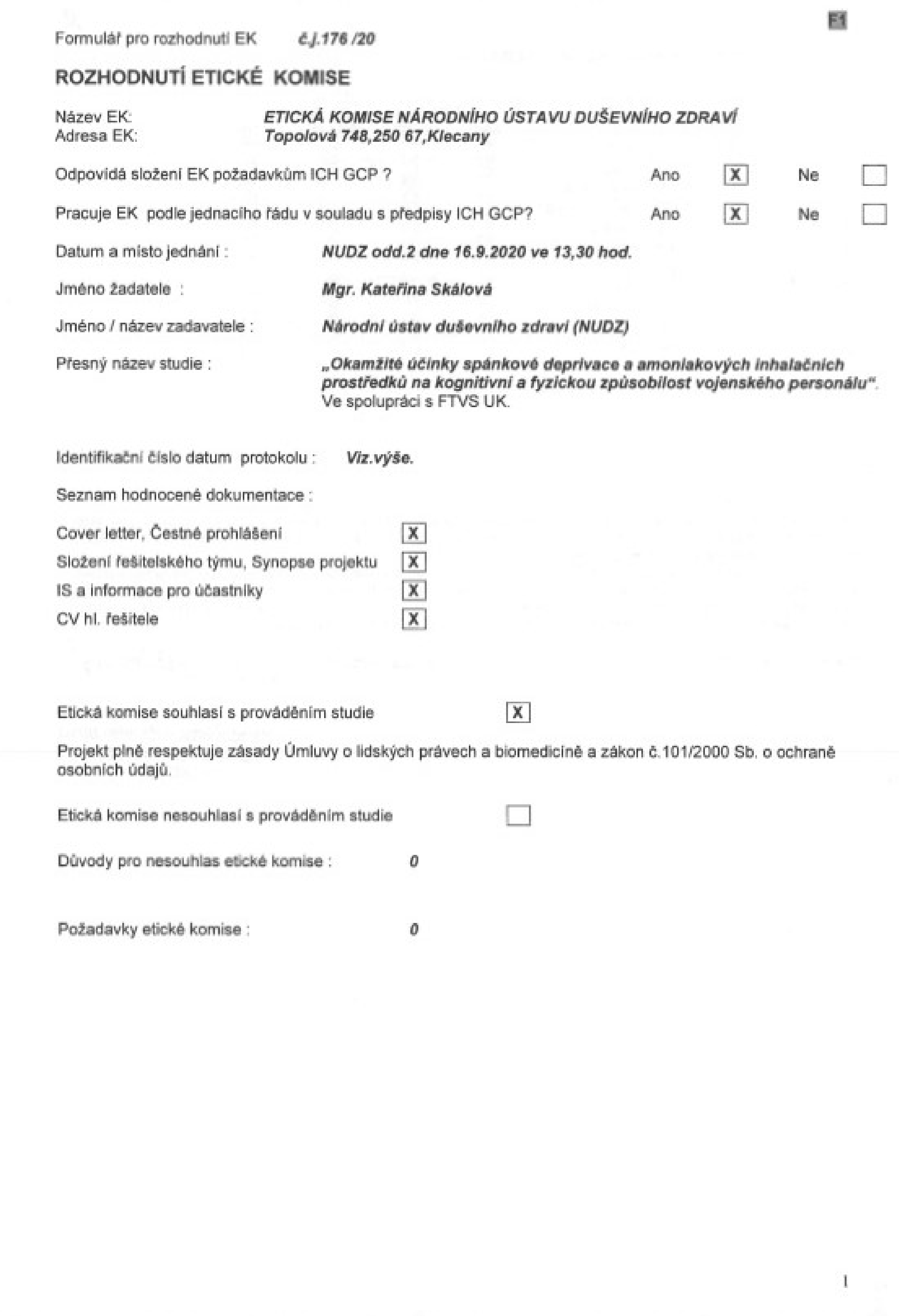
Jméno a příjmení osoby, která provedla poučení: Zdeněk Didek Podpis: ....

Prohlašuji a svým níže uvedeným vlastnoručním podpisem potvrzuji, že dobrovolně souhlasím s účastí ve výše uvedeném projektu a že jsem měl(a) možnost si řádně a v dostatečném čase zvážit všechny relevantní informace o výzkumu, zeptat se na vše podstatné týkající se účasti ve výzkumu a že jsem dostal(a) jasné a srozumitelné odpovědi na své dotazy. **Potvrzuji, že platnou výroční zdravotní prohlídku s hodnocením stupně A (nejvyšší stupeň zdravotní prohlídky v Armádě České republiky).** Byl(a) jsem poučen(a) o právu odmítnout účast ve výzkumném projektu nebo svůj souhlas kdykoli odvolat bez represí, a to písemně Etické komisi UK FTVS, která bude následně informovat předkladatele projektu. Dále potvrzuji, že mi byl předán jeden originál vyhotovení tohoto informovaného souhlasu.

Místo, datum ....

Jméno a příjmení účastníka: .... Podpis: ....

Appendix 9. The approved form by the Ethical Committee of The National Institute of Mental Health in Klecany.



F1

Formulář pro rozhodnutí EK *č.j.176 /20*

ROZHODNUTÍ ETICKÉ KOMISE

Název EK:	***ETICKÁ KOMISE NÁRODNÍHO ÚSTAVU DUŠEVNÍHO ZDRAVÍ***
Adresa EK:	***Topolová 748,250 67,Klecany***

Odpovídá složení EK požadavkům ICH GCP ? Ano ☒ Ne ☐

Pracuje EK podle jednacího řádu v souladu s předpisy ICH GCP? Ano ☒ Ne ☐

Datum a místo jednání :	***NUDZ odd.2 dne 16.9.2020 ve 13,30 hod.***
Jméno žadatele :	***Mgr. Kateřina Skálová***
Jméno / název zadavatele :	***Národní ústav duševního zdraví (NUDZ)***
Přesný název studie :	***„Okamžité účinky spánkové deprivace a amoniakových inhalačních prostředků na kognitivní a fyzickou způsobilost vojenského personálu“.*** Ve spolupráci s FTVS UK.

Identifikační číslo datum protokolu : ***Viz.výše.***

Seznam hodnocené dokumentace :

Cover letter, Čestné prohlášení ☒
Složení řešitelského týmu, Synopse projektu ☒
IS a informace pro účastníky ☒
CV hl. řešitele ☒

Etická komise souhlasí s prováděním studie ☒

Projekt plně respektuje zásady Úmluvy o lidských právech a biomedicíně a zákon č.101/2000 Sb. o ochraně osobních údajů.

Etická komise nesouhlasí s prováděním studie ☐

Důvody pro nesouhlas etické komise : ***0***

Požadavky etické komise : ***0***

Jednání etické komise se zúčastnili a hlasovali tito členové :

			Přítomen		Hlasoval	
			ANO	NE	ANO	NE
1.	Předseda :	Dr. Bareš	☒	☐	☒	☐
2.		Dr. Novák	☒	☐	☒	☐
3.		Mgr.Viktorinová	☐	☒	☐	☒
4.		Dr.Kratochvílová**MD**	☐	☒	☐	☒
5.		Bc.Sobotka	☐	☒	☐	☒
6.		Bc.Švejdová	☒	☐	☒	☐
7.		pí.Švecová	☒	☐	☒	☐
8.		p.Kuneš	☒	☐	☒	☐
9.		Dr. Andrashko	☒	☐	☒	☐
10.		Dr. Hejzlar	☐	☒	☐	☒
11.		Bc. Baslová	☒	☐	☒	☐

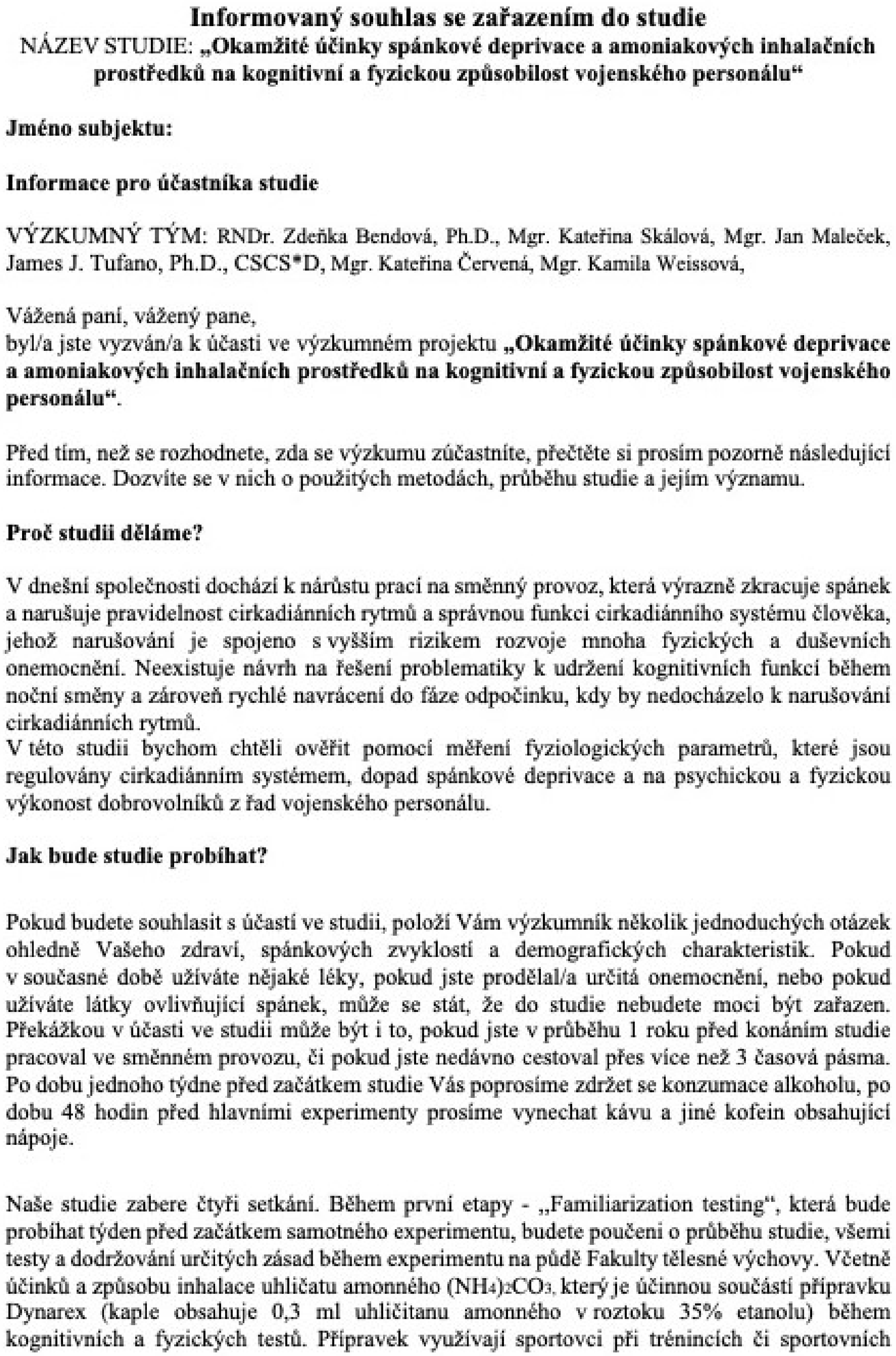
Etická komise upozorňuje žadatele na jeho povinnost zaslat k posouzení etické komisi všechny dodatky protokolu před jejich provedením. Výjimkou jsou dodatky, které jsou určeny k eliminaci bezprostředních rizik pro subjekty hodnocení a ty dodatky, které jsou administrativního charakteru - tyto musí být následně ohlášeny etické komisi.
Dále musí žadatel předložit k posouzení komisi skutečnosti, které zvyšují riziko subjektů hodnocení nebo výrazně ovlivňují průběh studie, ohlásit komisi všechny zaznamenané závažné neočekávané příhody, ohlásit komisi nové informace, které mohou negativně ovlivnit bezpečnost subjektů hodnocení nebo průběh klinického hodnocení a podat komisi zprávu o průběhu klinického hodnocení, a to jednou ročně v jeho průběhu a dále po jeho ukončení. Tyto údaje se zasílají předsedovi etické komise

Datum : 16.9.2020

podpis předsedy: doc. MUDr. Martin Bareš,
Ph.D.

Etická komise
Národní ústav duševního zdraví
Topolová 748, Klecany 250 67
tel.: 283 088 312

Appendix 10. The approved form of informed consent by the Ethical Committee of The National Institute of Mental Health in Klecany.



Informovaný souhlas se zařazením do studie

NÁZEV STUDIE: **„Okamžité účinky spánkové deprivace a amoniakových inhalačních prostředků na kognitivní a fyzickou způsobilost vojenského personálu“**

Jméno subjektu:

Informace pro účastníka studie

VÝZKUMNÝ TÝM: RNDr. Zdeňka Bendová, Ph.D., Mgr. Kateřina Skálová, Mgr. Jan Maleček, James J. Tufano, Ph.D., CSCS*D, Mgr. Kateřina Červená, Mgr. Kamila Weissová,

Vážená paní, vážený pane,
byl/a jste vyzván/a k účasti ve výzkumném projektu **„Okamžité účinky spánkové deprivace a amoniakových inhalačních prostředků na kognitivní a fyzickou způsobilost vojenského personálu“**.

Před tím, než se rozhodnete, zda se výzkumu zúčastníte, přečtěte si prosím pozorně následující informace. Dozvíte se v nich o použitých metodách, průběhu studie a jejím významu.

Proč studii děláme?

V dnešní společnosti dochází k nárůstu prací na směnný provoz, která výrazně zkracuje spánek a narušuje pravidelnost cirkadiánních rytmů a správnou funkci cirkadiánního systému člověka, jehož narušování je spojeno s vyšším rizikem rozvoje mnoha fyzických a duševních onemocnění. Neexistuje návrh na řešení problematiky k udržení kognitivních funkcí během noční směny a zároveň rychlé navrácení do fáze odpočinku, kdy by nedocházelo k narušování cirkadiánních rytmů.
V této studii bychom chtěli ověřit pomocí měření fyziologických parametrů, které jsou regulovány cirkadiánním systémem, dopad spánkové deprivace a na psychickou a fyzickou výkonost dobrovolníků z řad vojenského personálu.

Jak bude studie probíhat?

Pokud budete souhlasit s účastí ve studii, položí Vám výzkumník několik jednoduchých otázek ohledně Vašeho zdraví, spánkových zvyklostí a demografických charakteristik. Pokud v současné době užíváte nějaké léky, pokud jste prodělal/a určitá onemocnění, nebo pokud užíváte látky ovlivňující spánek, může se stát, že do studie nebudete moci být zařazen. Překážkou v účasti ve studii může být i to, pokud jste v průběhu 1 roku před konáním studie pracoval ve směnném provozu, či pokud jste nedávno cestoval přes více než 3 časová pásma. Po dobu jednoho týdne před začátkem studie Vás poprosíme zdržet se konzumace alkoholu, po dobu 48 hodin před hlavními experimenty prosíme vynechat kávu a jiné kofein obsahující nápoje.

Naše studie zabere čtyři setkání. Během první etapy - „Familiarization testing“, která bude probíhat týden před začátkem samotného experimentu, budete poučeni o průběhu studie, všemi testy a dodržování určitých zásad během experimentu na půdě Fakulty tělesné výchovy. Včetně účinků a způsobu inhalace uhličatu amonného $(NH_4)_2CO_3$, který je účinnou součástí přípravku Dynarex (kaple obsahuje 0,3 ml uhličitanu amonného v roztoku 35% etanolu) během kognitivních a fyzických testů. Přípravek využívají sportovci při trénincích či sportovních kompeticích. Při inhalaci přípravku dojde k okamžité aktivaci inhalačního reflexu, zrychlení dýchání, zvýšení srdeční frekvence a také napomáhá ke zvýšení bdělosti. Při dodržení všech předepsaných bezpečnostních předpisů (např. 15 cm vzdálenost aplikace od dýchacího ústrojí, dodržování doporučeného dávkování apod.) není látka nebezpečná pro zdravého jedince viz bezpečnostní list a vyjádření Etické komise UK FTVS.

Také budete vybaveni aktigrafem, přístrojem podobným hodinkám, určeným pro monitorování pohybové aktivity a intenzity okolního osvětlení. Aktigraf byste měli nosit nepřetržitě, pouze s výjimkou plavání a saunování, na zápěstí nedominantní ruky. Společně s aktigrafem obdržíte také senzor (čidlo) tělesné teploty (zařízení velikosti knoflíkové baterie s průměrem podobným korunové minci). Teplotní čidlo byste nosil připevněné na vnitřní straně bavlněného potítka tak, aby se dotýkalo zápěstí v místě, kde si lze nahmatat srdeční tep. Teplotní čidlo byste nosil nepřetržitě ve dne i v noci, stejně jako aktigraf, s výjimkou sprchování/koupání, plavání a saunování. Upevňování teplotního čidla je velmi snadné, jeho sundávání a opětovné nasazování Vám ukážeme. Nošení aktigrafu i teplotních čidel s sebou nenese žádná rizika. Obě zařízení budete nosit během studie i týden (dohromady 14 dní) po skončení pobytu ve spánkové laboratoři NUDZ. O jejich správném nošení budete znovu poučeni i během této etapy.

Během fáze „Baseline testing" se dostavíte ve čtvrtek ve večerních hodinách do spánkové laboratoře NUDZ, kde zůstanete zde až do nedělního rána. Bude Vám přidělen vlastní pokoj a během spánku Vám budou nasezeny elektrody v podobě speciální čepice pro účel polysomnografického vyšetření. Polysomnografické vyšetření slouží k získání záznamu elektrické aktivity Vašeho mozku a dalších biosignálů (pohyby očí, napětí svalů, aktivita) v průběhu spánku. V rámci vyšetření se ke snímání používají elektrody, které se umístí na hlavu, na místa definovaná mezinárodními standardy – jedná se zejména o vlasatou část hlavy a také o čelo. Elektrody budou zabudované ve speciální čepici. Aby signál mohl být zachycen, je nutné pod každou elektrodu vstříknout vodivý gel nebo vodivou pastu. Gel i pastu lze snadno umýt.

V pátek v dopoledních hodinách proběhnou hlavní tři testy (střelba laserem, fyziologické a kognitivní testy), které Vám byly demonstrovány při první fázi. Po ukončení testování se dostavíte do sesterny, kde proběhne jednorázový odběr krevních vzorků (pro pozdější stanovení hladiny glukózy a kortizolu). Odběr krve je standardním zdravotnickým výkonem používaným v medicíně a množství odebrané krve nepřesáhne 3 ml a ani po opakovaných odběrech nepředstavuje žádné zdravotní riziko. Krevní odběry se uskuteční dvakrát denně po 12 hodinách, a to ve 10h a 22h.

Po odběrech začnete dle harmonogramu (10h, 14h, 18h, 20h, 22h, 24h, 02h, 04h, 06h, 08h, 10h) odebírat sliny do speciálních označených zkumavek. Je důležité vyvarovat se konzumaci jídla alespoň 30 min před jedním odběrovým bodem. Na odběr všech vzorků a správný průběh dohlédne výzkumný pracovník.

Během těchto odběrů zůstáváte v přiděleném pokoji i během začátku poslední fáze experimentu. Ta spočívá v absenci spánku po dobu následující noci a dne. Spánková deprivace bude probíhat pod vlivem konstantních světelných podmínek. Bude Vám umožněno využívat PC a zařízení s LED displeji. V ranních hodinách opět podstoupíte stejné testy jako předchozí den akorát pod vlivem spánkové deprivace. Poté bude opět proveden jednorázový krevní odběr.

Během dalšího dne zůstanete znovu ve svých pokojích a během noci Vám bude umožněn spánek, ale monitorovaný polysomnografickým vyšetřením (stejnou metodou jako během čtvrteční noci). Následující den podstoupíte ráno poslední kognitivní testování a dále budete propuštěni ze spánkové laboratoře. Aktigrafy a teplotní čidla Vás poprosíme nosit ještě po dobu jednoho týdne, abychom mohli monitorovat případné změny.

Během dalších 2-3 týdnů po ukončení našich experimentů budete opět pozváni do spánkové laboratoře, kde proběhnou opětovná měření ve všech parametrech a spánková deprivace během kontrolovaných temnostních podmínek. V pokoji, kde se budete během noci zdržovat, bude slabé oranžové či červené světlo (intenzita pod 2 lux). Během temnostní fáze Vám již nebude umožněno používat zařízení emitující modré světlo nebo přístroje s technologii LED displejů. Na průběh a dodržování bdělého stavu bude dohlížet jeden z výzkumných pracovníků. V případě opuštění místnosti budete opatřeni oranžovými brýlemi, které zabrání osvícení nežádoucím světlem. Studie končí opět v neděli v dopoledních hodinách po provedení kognitivních testů a po dobu jednoho týdne si opět ponecháte aktigrafy s teplotními čidly.

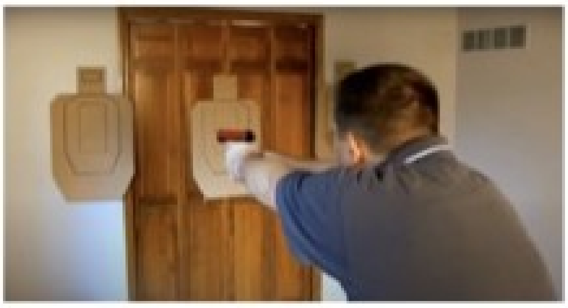
Popis kognitivních a fyzických testů

Kognitivní testy

Jedná se o počítačové testy, které mají za cíl měřit reakční čas a rozhodovací schopnosti. Před testováním střelby a výskoku podstoupíte 10 minutové kognitivní testování, které spočívá ve snaze reagovat co nejrychleji stiskem mezerníku na vizuální stimulus, který Vám bude v různých časových rozestupech promítán na monitor počítače. Během probdělé noci v rámci navození spánkové deprivace budete v různých časových intervalech podstupovat další podobné kognitivní testy na počítači, abychom zjistili změny parametrů v čase. Při spánkové deprivaci „ve tmě“ budou tyto testy prováděny s velmi slabým jasem monitoru a s nasazenými brýlemi blokujícími modré spektrum.

Testování střelby laserem

bude prováděno pomocí přenosné laserové střelnice. Během testování bude měřena přesnost střelby z makety služební zbraně s laserovým zaměřováním. Budete střílet do 3 různých terčů ve vzdálenosti 7 m od střelce. Vystřelíte celkem devětkrát (3 pokusy na každý terč). Poté se nadechnete čichací soli a zopakujete střelbu. Testování nepřekročí 10 minut.



Countermovement jump (CMJ): jde o skok, který měří výbušnou sílu. Postavíte se na silové desky (Kistler 141 9286BA, Kistler Instruments Inc, Winterthur, Switzerland) sloužící k měření inverzní dynamiky tj. reakci podložky na zatížení. Vaše postavení těla bude monitorovat lineární snímač polohy (Linear Positional Transducer, GymAware), který bude připojen k dřevěné tyči, kterou budete držet na ramenou. Provedete 3 CMJ a po inhalaci čichací soli dalších 6 skoků. Testování nepřekročí 10 minut.

Důvěrnost údajů

Pokud se studie zúčastníte, veškeré informace o Vás budou považovány za důvěrné. K informacím shromážděným v průběhu studie budou mít přístup odborní pracovníci Národního ústavu duševního zdraví a Etické komise Národního ústavu duševního zdraví v pseudoanonymizované formě. V žádné databázi nebude figurovat Vaše jméno. Vaše údaje budou zpracovávány a vedeny výhradně pod kódovým označeným. Propojení kódu s Vaším jménem může znát pouze výzkumní pracovníci.

Pokud budou výsledky studie prezentovány či publikovány v odborném tisku, bude to výhradně způsobem, aby nebylo možné určit žádné informace o konkrétním účastníku studie.

Svůj souhlas s použitím osobních údajů máte právo kdykoli odvolat zasláním písemného oznámení zkoušejícímu lékaři. Pokud svůj souhlas odvoláte, nebudete se moci studie nadále účastnit. Jestliže se však tak rozhodnete, nebudete nijak postižen/-a nebo znevýhodněn/-a v porovnání se situací před vstupem do studie.

Povinnost účasti ve studii:

Vaše účast ve studii je dobrovolná. Můžete odmítnout účast nebo můžete kdykoliv odstoupit bez udání důvodu, bez toho, že by to mělo vliv na péči, která je Vám poskytována. O ukončení účasti ve studii může rovněž rozhodnout zadavatel či Etická komise Národního ústavu duševního zdraví.

Zisk ze studie

Pokud se studie zúčastníte, přispějete k získání nových znalostí týkajících se vlivu spánkové deprivace lidský spánek a cirkadiánní rytmy. Účast ve studii je spojena s finanční odměnou 2000 Kč po úspěšném dokončení celé studie.

Protokol studie schválila Etická komise Národního ústavu duševního zdraví.
Máte-li jakékoliv dotazy ohledně samotné studie, obraťte se Mgr. Kateřinu Skálovou, mobil: 733640607, e-mail: katerina.skalova@nudz.cz

V případě dotazů ohledně etických aspektů výzkumu se můžete obrátit na jejího předsedu
Etická komise NUDZ, e-mail: ek@nudz.cz, tel. (+420) 283 088 312
NUDZ, lékař psychiatr – předseda EK: doc. MUDr. Martin Bareš, Ph.D.,

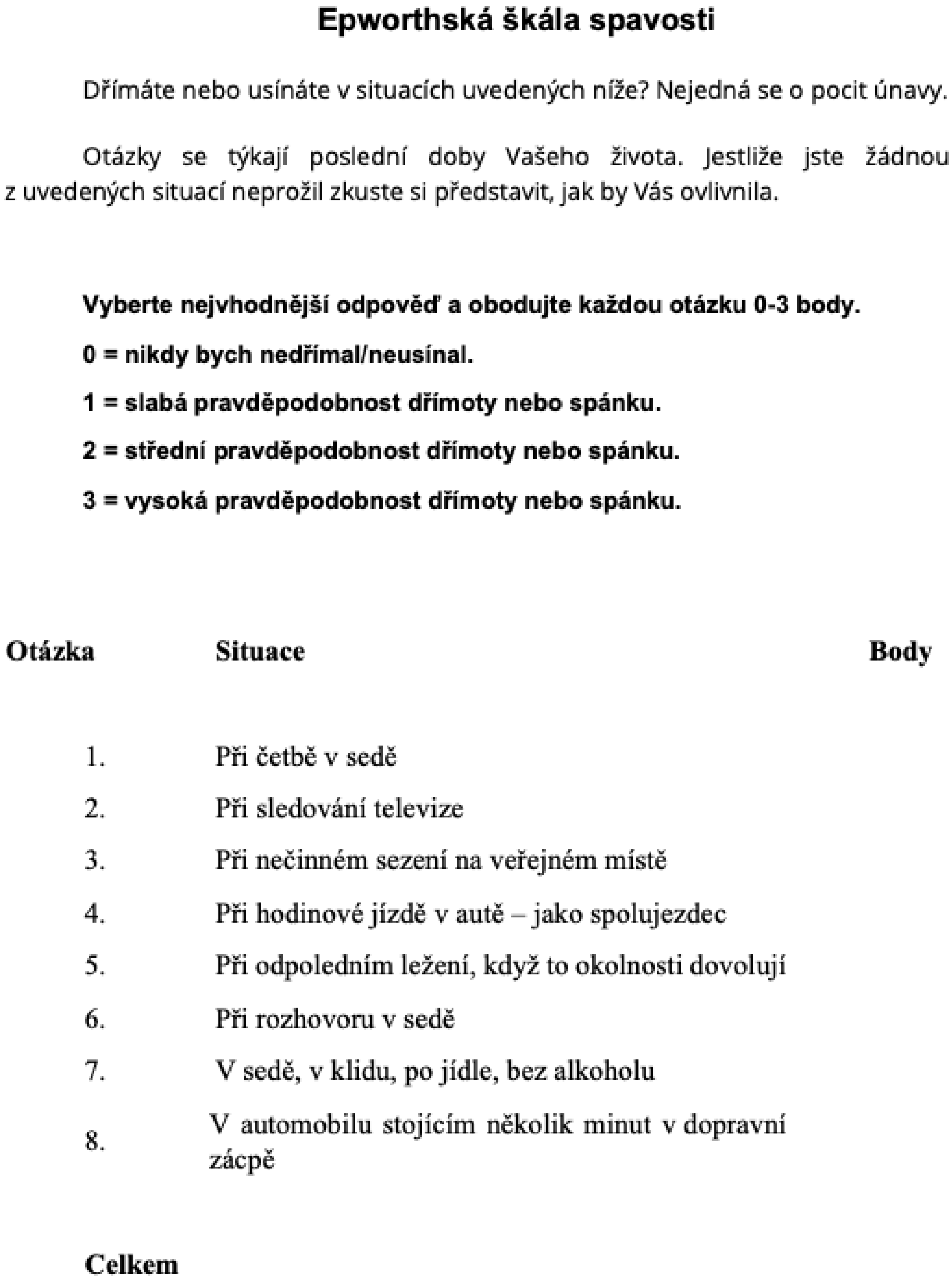
e-mail: martin.bares@nudz.cz, tel. (+420) 283 088 312

……………………………………… ………………………………………
Jméno a příjmení výzkumníka Datum, podpis

Informovaný souhlas
Svým podpisem stvrzuji, že jsem si přečetl/a výše uvedené informace, těmto informacím rozumím a dobrovolně souhlasím se svou účastí ve studii. Zároveň převezmu podepsaný stejnopis tohoto formuláře.

………………………………… ………………………………………
Jméno a příjmení účastníka studie Datum, podpis

Appendix 11. The Epworth Sleepiness Scale used during the research.



Epworthská škála spavosti

Dřímáte nebo usínáte v situacích uvedených níže? Nejedná se o pocit únavy.

Otázky se týkají poslední doby Vašeho života. Jestliže jste žádnou z uvedených situací neprožil zkuste si představit, jak by Vás ovlivnila.

Vyberte nejvhodnější odpověď a obodujte každou otázku 0-3 body.

0 = nikdy bych nedřímal/neusínal.

1 = slabá pravděpodobnost dřímoty nebo spánku.

2 = střední pravděpodobnost dřímoty nebo spánku.

3 = vysoká pravděpodobnost dřímoty nebo spánku.

Otázka	**Situace**	**Body**
1.	Při četbě v sedě	
2.	Při sledování televize	
3.	Při nečinném sezení na veřejném místě	
4.	Při hodinové jízdě v autě – jako spolujezdec	
5.	Při odpoledním ležení, když to okolnosti dovolují	
6.	Při rozhovoru v sedě	
7.	V sedě, v klidu, po jídle, bez alkoholu	
8.	V automobilu stojícím několik minut v dopravní zácpě	
Celkem		

Appendix 12. Borg Category-Ratio 10 scale.