Zbornik 17. mednarodne multikonference

INFORMACIJSKA DRUŽBA – IS 2014

Zvezek I

Proceedings of the 17th International Multiconference

INFORMATION SOCIETY – IS 2014

Volume I

Okoljska ergonomija in fiziologija
Environmental Ergonomics and Physiology

Uredila / Edited by
Tadej Debevec, Igor B. Mekjavič

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9. oktober 2014 / October 9th, 2014
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PREDGOVOR MULTIKONFERENCI INFORMACIJSKA DRUŽBA 2014

Multikonferenca Informacijska družba (http://is.ijs.si) s sedemnajsto zaporedno prireditvijo postaja tradicionalna kvalitetna srednjeevropska konferenca na področju informacijske družbe, računalništva in informatike. Informacijska družba, znanje in umetna inteligenca se razvijajo čedalje hitreje. Čedalje več pokazateljev kaže, da prehajamo v naslednje civilizacijsko obdobje. Npr. v nekaterih državah je dovoljena samostojna vožnja inteligentnih avtomobilov, na trgu pa je moč dobiti kar nekaj pogosto prodajanih tipov avtomobilov z avtonomnimi funkcijami kot »lane assist«. Hkrati pa so konflikti sodobne družbe čedalje bolj nerazumljivi.

Letos smo v multikonferenco povezali dvanajst odličnih neodvisnih konferenc in delavnic. Predstavljenih bo okoli 200 referatov, prireditev bodo spremljale okrogle mize, razprave ter posebni dogodki kot svečana podelitev nagrad. Referati so objavljeni v zbornikih multikonference, izbrani prispevki bodo izšli tudi v posebnih številkah dveh znanstvenih revij, od katerih je ena Informatica, ki se ponaša s 37-letno tradicijo odlične evropske znanstvene revije.

Multikonferenco Informacijska družba 2014 sestavljajo naslednje samostojne konference:

- Inteligentni sistemi
- Izkopavanje znanja in podatkovna skladišča
- Sodelovanje, programska oprema in storitve v informacijski družbi
- Soočanje z demografskimi izzivi
- Vzgoja in izobraževanje v informacijski družbi
- Kognitivna znanost
- Robotika
- Jezikovne tehnologije
- Interakcija človek-računalnik v informacijski družbi
- Prva študentska konferenca s področja računalništva
- Okolijska ergonomija in fiziologija
- Delavnica Chiron.

Soorganizatorji in podporniki konference so različne raziskovalne in pedagoške institucije in združenja, med njimi tudi ACM Slovenija, SLAIS in IAS. V imenu organizatorjev konference se želimo posebej zahvaliti udeležencem za njihove dragocene prispevke in priložnost, da z nami delijo svoje izkušnje o informacijski družbi. Zahvaljujemo se tudi recenzentom za njihovo pomoč pri recenziranju.

V 2014 bomo drugič podelili nagrado za življenjske dosežke v čast Donalda Michija in Alana Turinga. Nagrado Michie-Turing za izjemen življenjski prispevek k razvoju in promociji informacijske družbe je prejel prof. dr. Janez Grad. Priznanje za dosežek leta je pripadlo dr. Janezu Demšarju. V letu 2014 četrtič podeljujemo nagrado »informacijska limona« in »informacijska jagoda« za najbolj (ne)uspešne poteze v zvezi z informacijsko družbo. Limono je dobila nerodna izvedba piškotkov, jagodo pa Google Street view, ker je končno posnel Slovenijo. Čestitke nagrajencem!

Niko Zimic, predsednik programskega odbora
Matjaž Gams, predsednik organizacijskega odbora

FOREWORD - INFORMATION SOCIETY 2014

The Information Society Multiconference (http://is.ijs.si) has become one of the traditional leading conferences in Central Europe devoted to information society. In its 17th year, we deliver a broad range of topics in the open academic environment fostering new ideas which makes our event unique among similar conferences, promoting key visions in interactive, innovative ways. As knowledge progresses even faster, it seems that we are indeed approaching a new civilization era. For example, several countries allow autonomous card driving, and several car models enable autonomous functions such as "lane assist". At the same time, however, it is hard to understand growing conflicts in the human civilization.

The Multiconference is running in parallel sessions with 200 presentations of scientific papers, presented in twelve independent events. The papers are published in the Web conference proceedings, and a selection of them in special issues of two journals. One of them is Informatica with its 37 years of tradition in excellent research publications.

The Information Society 2014 Multiconference consists of the following conferences and workshops:

- Intelligent Systems
- Cognitive Science
- Data Mining and Data Warehouses
- Collaboration, Software and Services in Information Society
- Demographic Challenges
- Robotics
- Language Technologies
- Human-Computer Interaction in Information Society
- Education in Information Society
- 1st Student Computer Science Research Conference
- Environmental Ergonomics and Psysiology
- Chiron Workshop.

The Multiconference is co-organized and supported by several major research institutions and societies, among them ACM Slovenia, SLAIS and IAS.

In 2014, the award for life-long outstanding contributions was delivered in memory of Donald Michie and Alan Turing for a second consecutive year. The Programme and Organizing Committees decided to award the Prof. Dr. Janez Grad with the Michie-Turing Award. In addition, a reward for current achievements was pronounced to Prof. Dr. Janez Demšar. The information strawberry is pronounced to Google street view for incorporating Slovenia, while the information lemon goes to cookies for awkward introduction. Congratulations!

On behalf of the conference organizers we would like to thank all participants for their valuable contribution and their interest in this event, and particularly the reviewers for their thorough reviews.

Niko Zimic, Programme Committee Chair
Matjaž Gams, Organizing Committee Chair

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PREDGOVOR

Veseli nas, da bo v okviru letošnje, že 17. Multikonference Informacijska družba potekal tudi prvi simpozij z naslovom Okoljska ergonomija in fiziologija (OEF). Četudi se na prvi pogled morda zdi, da simpozij ne sodi v okvir Mulitkonference, sta interdisciplinarna povezanost tem simpozija in vpliv znanosti o okoljski ergonomiji na družbo kot celoto zagotovili, da ima tudi ta tematika svoje mesto znotraj Informacijske družbe. Veliko število ljudi je vsakodnevno izpostavljenih različnim okoljskim dejavnikom, tako med športnimi in rekreacijskimi dejavnostmi, kot tudi med delom. Problemi s katerimi se to, vseskozi naraščujoče, število posameznikov srečuje in njihovo ustrezno reševanje bodo glavni fokus simpozija. Tri podpodročja okoljske ergonomije, ki jim bomo na simpoziju posvetili glavno pozornost so aplikativna športna fiziologija, termoregulacija in simulirana breztežnost.

Poleg zgoraj navedenega, bo eden od glavnih ciljev simpozija vzpostavitev platforme za mreženje in izmenjavo znanj med lokalnimi in mednarodnimi raziskovalci, kot tudi pretok znanj od izkušenih raziskovalcev, do tistih, ki so trenutno šele na začetku svoje znanstvene poti. Upamo, da bo simpozij ponudil uporabne in praktične informacije tako raziskovalcem, kot tudi industrijskim partnerjem in zainteresirani javnosti. Vljudno vabljeni!

Tadej Debevec in Igor B. Mekjavič, predsednika konference

PREFACE

We are delighted that the 17th Information Society Multiconference (October 2014) will host the inaugural Environmental Ergonomics and Physiology symposium (EEP). Although, at first glance, the topic of the symposia might be outside of the Multiconference scope, the inter-disciplinarity of the symposia topics and the effects of environmental ergonomics related science on the society as a whole warrant its place among the sub-conferences. Vast amount of people is nowadays daily exposed to different acute and chronic environmental stressors for sporting, recreational and occupational pursuits. The problems that this growing number of individuals face, and how to effectively address them, will be the main focus of the EEP symposia. The main three sub-areas of environmental ergonomics that will be addressed during the EEP include applied exercise physiology, thermoregulation and simulated microgravity.

One of the key aims of the inaugural EEP symposia is to provide a platform for networking and knowledge exchange, connecting regional and international as well as early-stage and experienced scientists. We hope the symposia will provide useful and valuable information to the researchers, industrial partners as well as to the interested public. Kindly invited!

Tadej Debevec in Igor B. Mekjavič, Conference chairs

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THERMOREGULATORY RESPONSES AND HYDRATION STRATEGIES DURING EXERCISE IN THE YOUTH: A REVIEW

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ABSTRACT

This paper presents a review of the scientific literature about thermoregulation and hydration during exercise in young athletes. Many sports events are performed in environmental conditions in which thermal strain could represent a threatening situation for health and performance of the young participants. Growth and maturation produce several anatomical and physiological alterations in the organism, leading to different patterns of heat dissipation and behavioral responses. Age-related differences, as sweat glands activity and vasomotor responses, have been reported by several authors. These results highlight relevant factors that not only the trainers, but also sports events organizers should carefully consider.

1 INTRODUCTION

Maintenance of thermoregulation and fluid homeostasis is vital for the organism and represents a challenge during physical activity. Moderate levels of dehydration, usually considered as > 2% of body mass loss, can impair thermoregulatory responses and reduces cardiac output [1] Furthermore, dehydration as indicated above could impair sports specific performance and skills [2]. Growth and maturation induce several biological and physiological alterations in the organism, and thermoregulation is evenly affected by it. Children have anatomical and physiological characteristics different from adults, as body composition, water, and bone density [3]. In particular, children have a higher ratio between surface area and body mass. This value represents a better heat transfer through dry pathways (i.e., conduction, convection and radiation), which can result in a greater increase in body temperature when environmental temperature is higher than skin temperature [3, 4]. Furthermore, sweat response seems to be reduced in children decreasing the ability for evaporative heat loss [3]. These differences require a better knowledge of physiological adaptations to maintain thermal and fluid balance, in order to reduce the risk of injuries and enhance performance in different environments. This paper aims to resume some of these findings and suggest the best strategies for children and adolescents exercising in the heat.

2 THERMAL BALANCE IN THE YOUTH

Thermal balance is achieved through several physiological adaptations in response to different rates of heat storage or loss, resulting in different body temperatures. These responses are mainly related to changes in blood flow and vasomotor alterations, and evaporative heat loss. In particular, evaporative loss is the main mechanism for heat dissipation when environmental temperature is high. As discussed above, children sweat production is reduced when compared to adults. The cause of this reduction is not related to the total number of sweat glands, since it is fixed between 2 and 3 years of age [5]. Thus, the increased sweat rate during growth and maturation is observed as a consequence of the increased number of glands activated by heat, the increased amount of sweat output per gland (because of bigger sweat glands), and their combination [5]. Conversely, different results suggest that children possess a better sweating efficiency, maybe because of the less widespread sweat drops and the consequent enhanced evaporation from the skin. Additionally, larger sweat drops could drop off the skin, without any cooling effect [6]. In this study the authors observed that in prepubertal boys there was a lower percentage of sweat that was not evaporated than in young adults or elderly, and in a hot dry condition the first were the most efficient thermoregulators [6]. Growth related increase in sweat rate is not equal through the whole body, since regional differences are observed markedly when exercise intensity is high. In detail, reduced sweat rates are observed on chest, thigh and forehead [4]. Differences in hormones concentrations, in particular testosterone and prolactin may affect sweat gland function and sweat composition [7]. Conversely, due to the greater surface area per unit of body mass, the increased cutaneous vasodilation and blood flow to the skin [4], children can transfer the heat better through conduction, convection and radiation [3]. Prepubertal children show a greater change in skin blood perfusion per change in core temperature than in young adults [8]. In children, energy cost of movement is greater than in adults because of a reduced efficiency of locomotion, in particular on treadmill. Thus, the amount of metabolic heat produced is greater than in adults [9, 10]. Despite the lower sweat rate, children seem to be well capable to compensate the heat storage through other mechanisms, defining them as efficient thermoregulators [9, 10, 11]. In table 1 are summarized children characteristics and its effects on thermoregulation.

CHARACTERISTICS	EFFECTS
Greater energy cost of locomotion	Greater metabolic heat storage
Smaller sweat gland size	Smaller secretory portion and reduced sweat rate
Reduced hormonal sensitivity and concentration	Reduced sweat rate
Reduced sweat glands cholinergic stimulus	Reduced sweat rate
Lower cardiac output	Increased cardiovascular strain
Increased cutaneous vasodilation	Increased dry pathways heat transfer
Greater body surface to body mass ratio	Increased dry pathways heat transfer

Table 1: children thermoregulatory characteristics and effects

3 FLUID BALANCE IN THE YOUTH

As discussed before, also moderate level of dehydration as 2% of body mass loss (BM) can induce detrimental effects in the organism. These effects are a reduced plasma volume, an increase in plasma osmolality, greater increase in core temperature and heart rate, and reduced sweat rate with severe hypohydration [12]. In detail, the increased plasma osmolality seems to be the main factor reducing sweat rates, instead of hypovolemia [12]. In adults, sweat is hypotonic in relation to blood plasma. In children and adolescents sweat is even more hypotonic, slightly preventing from hyponatremia and hypokalemia [13]. Thus, due to the different volumes of water and electrolytes losses during exercise, specific plans for fluid replacements need to be proposed for the youngster. Moderate levels of hypohydration seem to also negatively affect sport specific skills. When hypohydration of 2% of BM was achieved during soccer performance, the total distance covered in a Yo Yo Intermittent Recovery Test was significantly reduced [14]. However, the authors could not identify the cause of this reduction as effect of physiological alterations rather than the increased psychological stress. Maintaining euhydration during physical activity is not the only important factor for fluid homeostasis, since euhydration is not usually achieved even before starting training session. Through measurements of morning urine samples from different adolescents athletes of different sports, indicated that the great part of them (over 89%) was already slightly hypohydrated (USG $\geq$ 1020 mg/dL) [15]. These values, even if moderately reduced, were in mean evenly greater than 1020 mg/dL immediately before athletes' training session. Exception made for swimmers, who possessed greater USG values after training than before, all the athletes were affected by a higher degree of dehydration as effect of their physical activity [15]. In the same study, the authors found a mean BM loss of 1.1±0.07% after approximately 90 min of training in warm temperatures, both indoor and outdoor. This amount of water losses seems not to be particularly detrimental for normal physiological functions (as discussed above); however, since they were already in a hypohydrated condition, even 1% of BM loss could represent a moderate threaten to health and performance [15]. Even when fluids are available and athletes are allowed to drink ad libitum, both children and adults are hypohydrated after training, resulting in voluntary dehydration [16].

4 YOUNG ATHLETES EXERCISING IN THE HEAT: PRACTICAL SUGGESTIONS

As observed above, several changes in thermoregulation and fluids balance countersign growth and maturation, both on physiological and psychological factors. These differences could require specific observations and protocols when coaching or organizing events in which children and adolescents are involved. Major consensus identify children as efficient thermoregulators as adults, even if throughout different mechanisms. It is suggested that these differences in thermoregulation do not affect physical performance, as observed in the exhaustion time at steady-load cycling, in both cool and hot environments, between prepubertal boys and young adults [10]. In the same study, the authors observed no significantly different increase between groups in heart rate, rectal temperature and cardiac index during exercise. As for the adults, one factor affecting exhaustion time seems to be the core temperature. In both cold and hot climates, core temperature at exhaustion time was similar, indicating that the greater rate of rise of the temperature in the hot condition conduced to the shorter duration of the exercise [17]. From the physiological point of view, we could suggest to use the same protocols and procedures regarding the environmental conditions that are commonly used with the adults. However, it is relevant that exertional heatstroke is the leading cause of preventable death in youth sports [18]. Since physiological thermoregulatory responses seem not to be different from adults, possible explanations could be attributed to behavioral thermoregulation. There is a lack of information about behavioral thermoregulation in the youth, but differences in perceived exertion and thermal strain, cumulative experience, cognitive development and decision-making capacity may subject the young athlete to sub-par performance or greater risk of thermal injuries [19]. Better thermoregulatory responses could be achieved through heat acclimation. In adults, different protocols have been studied and its effects have been evaluated. For the pediatric population there is a lack of heat acclimation guidelines [20], even if it suggested that in prepubertal boys age related factors associated with the thermoregulatory system prevent them from deriving full effectiveness of exercise-in-heat acclimatization protocols [21].

If thermal factors seem to not be relevantly different depending on age factors, maintaining fluid balance requires specific knowledge when children and adolescents are involved. Results suggest that the youngster smaller levels of dehydration induce a higher rate of increase in core temperature [22]. As a consequence, it highlights the need of well-defined guidelines for fluids and electrolyte replacement to prevent detrimental effects on performance and health. Total sweat losses during exercise are smaller in prepubertal boys than in young adults, as discussed above. It depends on the exercise intensity, environmental conditions and subjects variability; total sweat rate in prepubertal boys can be about half the sweat rate measured in young adults in the same condition [6]. Sweat electrolytes composition is also different depending on age factors. In prepubertal boys, Na^+ and Cl^- in sweat are 33.0±2.2 mmol/L and 22.0±1.8 mmol/L, respectively. Differently, in young adults Na^+ and Cl^- are 50.0±8.0 mmol/L and 40.0±7.5 mmol/L, respectively. Differences for gender are observable in both age groups, in which females showed smaller values. Sweat K^+ concentration, instead, is greater in the youngster and in females [23]. Based on these results, defining beverages' fluids and electrolytes composition should be specifically designed for the youngster. Sodium, potassium and other electrolytes are relevant for maintaining physiological functions and health, as additional carbohydrates (CHOs) could prevent glucose depletion and fatigue. However, increasing the concentration of carbohydrates would reduce the rate of gastric emptying and fluids absorption [24]. Sodium implementation would maintain high plasma osmolality, stimulating thirst, keeping extracellular Na^+ concentration and enhancing water and CHOs absorption from the small intestine [25]. However, great amounts of sodium would reduce beverages' patability. Since dehydration is usually voluntary, it is fundamental to find the best flavors to induce children to drink, as shown with an increase of 91% more fluids consumed if grape-flavored [26]. Educational interventions about modification of hydration behaviors in the youngster are not successful; to reduce the risk of dehydration during exercise the best strategy is to prescribe individualized hydration protocols and scheduling appropriate rest and water breaks [27].

5 CONCLUSION

In this paper we wanted to resume the main findings on thermoregulation and hydration practices in young athletes. Although sweat rate in the youngster is relevantly lower than in adults, through different mechanisms children are as efficient thermoregulators as adults are. Cognitive factors associated with heat perception are not deeply studied in the pediatric population, do not providing sufficient knowledge on this thermoregulatory aspect. Fluid and electrolyte losses through sweat are different in the youngster and in the adults, and in prepubertal boys even small levels of hypohydration can affect thermoregulatory functions. Drinking ad libitum is not sufficient to allow an appropriate fluids replacement, thus it is suggested to propose specific and individualized strategies for hydration in the pediatric population.

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THE EFFECT OF GENDER ON REGIONAL THERMAL COMFORT

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Background This study determined the whole body and regional (head, hands, feet, arms, legs, front torso and back torso) thermal comfort zone (TCZ) in males and females.

Methods Eight male and eight female subjects completed eight trials, performed in a climate chamber maintained at 33 °C and 30% relative humidity. During each trial, the subjects donned a water-perfused suit and assumed a semi-supine position. The temperature of the water perfusing the suit changed in a sinusoidal manner from 10° to 50°C. Subjects were instructed that they could control the magnitude of the temperature oscillations (ie. heating or cooling) by depressing a switch. Over the one hour period of each trial, the temperature of the water perfusing the suit described a damped oscillation due to the subjects' regulation of thermal comfort. The width of this damped temperature oscillation was defined as the thermal comfort zone (TCZ). In this manner the TCZ of several body regions (arms, legs, front torso, back torso and whole body) was assessed. In each trial subjects controlled the temperature of a different body region, while the rest of the suit temperature was maintained at 33 °C. The thermal comfort of hands, feet and head was also studied with subjects exposed directly to ambient conditions, wearing only underwear and water perfused gloves, socks or hood, depending on the peripheral region studied. During each trial, heart rate, rectal temperature, skin temperature and skin heat flux were recorded at minute intervals, while the subjects also provided ratings of thermal comfort and sensation every 10 min.

Results Maximal regulated temperature was significantly higher ($p<0.05$) in female subjects, who preferred higher suit temperature when regulating TCZ for the legs and front torso, while the difference in minimal suit temperatures between genders was not significant.

Conclusion The thermal comfort zone was greater in female subjects, because of a significantly higher maximal comfort temperature, whereas the minimal comfort temperature was no significantly different from that observed in males.

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SEASONAL EFFECTS ON AUTONOMIC AND BEHAVIOURAL TEMPERATURE REGULATION

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Background As part of a study investigating the effect of nonthermal factors (ie. hypoxia and inactivity) on temperature regulation, subjects were required to conduct several trials over a period of one year. To account for seasonal effects on the observed responses to the thermal stimuli, we investigated the characteristics of behavioural and autonomic temperature regulation during this period.

Methods Twelve subjects participated in three trials conducted in October 2012 (T1), April (T2) and October 2013 (T3). Each trial comprised two separate studies. In one (behavioural temperature regulation study, BTR), subjects donned a water-perfused suit, in which the temperature of the water perfusing the suit changed in a sinusoidal manner from 10° to 50°C (Yogev et al., 2010). Subjects were instructed to maintain the temperature of the suit within a thermally comfortable range by depressing a switch. Over the 1-hr period of each trial, the temperature of the water perfusing the suit described a damped oscillation due to the subjects' regulation of thermal comfort. The width of this damped temperature oscillation was defined as the thermal comfort zone (TCZ). In the second trial (autonomic temperature regulation, ATR), subjects exercised on a cycle ergometer to elevate their core temperature by approx. 1°C, thus initiating the responses of sweating and vasodilatation. Thereafter they were immersed in a bath of stirred water maintained at 28°C (Mekjavic and Sundberg, 1992). During the 2-hr period of immersion we determined the core temperature thresholds for the cessation of sweating, onset of vasoconstriction, and onset of shivering. During the BTR study we monitored skin and rectal temperature at minute intervals and recorded subjects' ratings of thermal comfort and thermal sensation every 10 min. In the ATR study we additionally measured sweating rate and peripheral perfusion, as reflected in the forearm-fingertip skin temperature gradient.

Results TCZ varied from 6 °C in T1, to 8.3 °C in T2 and 11.4 °C in T3. The difference between T1 and T3 being significantly different. There were no significant differences in the measured autonomic thermoregulatory responses between trials T1, T2 and T3.

Conclusion The differences observed in the BTR trials between T1 and T3 were for trials conducted during the same season. No differences in autonomic thermoregulatory responses were observed between the three trials. We conclude that there are no seasonal influences on behavioural and autonomic temperature regulation.

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EFFECTS OF EXERCISE TRAINING DURING NORMOBARIC HYPOXIC CONFINEMENT ON HORMONAL APPETITE REGULATION AND OXIDATIVE STRESS

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Background High altitude sojourns are frequently associated with weight loss and increased physical activity levels. Both, hypoxia and exercise have been shown to influence appetite and oxidative status. We aimed to discern the effects of moderate exercise training during continuous hypoxic exposure on select markers of hormonal appetite regulation and oxidative stress.

Methods Fourteen healthy males participated in a continuous 10-day exposure to normobaric hypoxia (P_IO_2=88.2±0.6 mmHg; simulated altitude ~4000 m) either combined with moderate intensity exercise training (Exercise group, n=8; two 60-min exercise sessions·day^{-1} at 50% of altitude-specific maximal aerobic power) or without any exercise (Sedentary group, n=6). To quantify fasting and postprandial plasma concentrations of selected appetite-related hormones a meal tolerance test was performed before (Pre) and just prior to the end of confinement (Post). The oxidative stress and antioxidant markers were assessed at Pre, Post and 24-hrs after confinement cessation (Post+1).

Results No differences between Pre and Post or between groups were noted in fasting and postprandial concentrations of total ghrelin, peptide YY, glucagon-like peptide-1, and leptin. Augmented advanced oxidation protein products and nitrotyrosine levels at Post indicate increased oxidative stress levels in the Sedentary group only. On the other hand, antioxidant markers (superoxide dismutase and catalase) were only increased at Post in the Exercise group ($p<0.05$). Ferric-reducing antioxidant power was higher in the Exercise compared to the Sedentary group at Post+1 ($p<0.05$).

Conclusion Daily moderate intensity exercise training during 10-day hypoxic confinement does not seem to alter hormonal appetite regulation but can attenuate hypoxia-induced oxidative stress in healthy young individuals.

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BODY COMPOSITION MODULATION DURING AND FOLLOWING SIMULATED PLANETARY HABITATION: THE PLANHAB STUDY

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ABSTRACT

Envisaged planetary habitats will expose astronauts to simultaneous microgravity and hypoxia. We aimed to investigate whether hypoxia exacerbates reductions in body mass observed during simulated microgravity-induced unloading.

To examine the effects of simulated microgravity and hypoxia, eleven healthy males underwent three 21-day confinements in a randomized fashion: 1) normoxic bed rest (NBR; F_iO_2=0.209; P_iO_2=133.1±0.3) 2) hypoxic ambulatory confinement (HAMB; F_iO_2=0.141±0.004; P_iO_2=90.0±0.4; ~4000 m), and 3) hypoxic bed rest (HBR; F_iO_2=0.141±0.004; P_iO_2=90.0±0.4). A standardized, individually adjusted, 14-day dietary menu was applied and rotated in all campaigns. Body mass was measured daily. Whole body and regional body composition was determined before and after the campaigns using dual-energy X-ray absorptiometry.

Energy intakes were significantly lower than targeted (NBR:-5%; HAMB:-14%; HBR:-6%). Body mass was significantly reduced following all campaigns (NBR:-3%; HAMB:-4%; HBR:-5%). Whole body fat free mass was reduced (NBR:-4%; HAMB:-5%; HBR:-5%), secondary to lower limb fat free mass reduction following all campaigns. Whole body fat mass was not significantly altered.

In conclusion, simulated altitude of ~ 4000 m does not seem to exacerbate the whole body mass and fat free mass reductions following a 21-day bed rest.

1 BACKGROUND

Microgravity-induced unloading can result in decreased body weight, secondary to reduced postural muscle mass [1]. To minimize the risk of decompression sickness of astronauts preparing for extravehicular activities on the Moon or Mars surface the envisaged planetary habitats will be hypobaric and hypoxic [2].

Weight loss is also frequently reported following high altitude sojourns [3-5]. This altitude related weight loss has been attributed to "altitude anorexia" which is not completely understood. Main suggested mechanisms include energy imbalance (reduced intake and increased expenditure) and appetite alterations [6,7]. Even though some controlled laboratory studies suggest that ambiental hypoxia reduces resting energy expenditure (REE) [8], evidence from well-controlled field studies show that REE might increase as a result of prolonged hypoxic exposures [9]. Reductions in appetite are also often reported during and following ascents to high altitudes. Although initially linked to acute mountain sickness (AMS) and lack of palatable food, the persistence of decreased appetite after AMS symptoms abate [4], imply that hypoxia *per se* might influence appetite.

Few studies to date investigated the combined effects of simulated microgravity and hypoxia [10-12]. Both Stevens et al. [10] and Loeppky et al. [11] did not find any significant changes in body mass or composition due to hypoxia *per se*. The lack of significant body mass changes in both aforementioned studies could be attributed to the shortage of proper nutritional control and the *ad libitum* feeding strategy. We also recently [12], failed to identify any specific effect of hypoxia following a 10-day bed rest intervention. We reasoned that the exposure duration was insufficient to result in significant body composition alterations which are typically reported following altitude exposures lasting a few weeks or more [5]. Accordingly, this study aimed to investigate the combined and separate effects of prolonged hypoxia and unloading on body mass and composition. We hypothesized that exposure to hypoxia will exacerbate bed rest induced body mass reduction.

2 METHODS

This study was part of a larger research programme investigating the physiological and psychological effects of simulated planetary habitation on healthy humans (PlanHab: Planetary Habitat simulation study). The study protocol was approved by the National Committee for Medical Ethics of the Republic of Slovenia.

2.1 Participants

Eleven healthy, near sea level residents finished all three campaigns. Their baseline characteristics were as follows: Age = 27 ± 6 yrs; body mass = 76.7 ± 11.8 kg; stature = 179 ± 3 cm; BMI = 23.7 ± 3.0 kg·m^{-2}; body fat= 21 ± 5%; maximal oxygen uptake = 44.3 ± 6.1 mL·kg^{-1}·min^{-1}.

2.2 Study outline

The participants underwent three 21-day confinements in a cross-over designed and counterbalanced manner: 1) Normoxic bed rest to determine the effects of unloading (NBR; fraction of inspired O_2 (F_iO_2) = 0.209; partial pressure of inspired oxygen (P_iO_2) = 133.1 ± 0.3) 2) Hypoxic ambulatory confinement to determine the effect of hypoxia (HAMB; F_iO_2 = 0.141 ± 0.004; P_iO_2 = 90.0 ± 0.4; ~ 4000 m simulated altitude) and 3) Hypoxic bed rest to determine the combined effects of both factors (HBR; F_iO_2 = 0.141 ± 0.004; P_iO_2 = 90.0 ± 0.4; ~ 4000 m simulated altitude). All campaigns were performed at the Olympic Sport Centre Planica, Slovenia (940 m). Campaigns lasted 32 days for each individual participant and had three distinct phases: i) the initial testing phase (Pre) to obtain the baseline measurements, ii) the 21-day confinement phase (Day 1 – Day 21) during which the participants were exposed to their designated condition (NBR, HAMB & HBR), and iii) the recovery phase that enabled the researchers to obtain the post-confinement measurements (Post). Additional tests were also performed 14 days after the end of each confinement period (Rec).

The environmental conditions within the hypoxic facility were controlled and remained fairly stable during the campaigns (ambient temperature = 24.4 ± 0.7 ˚C; relative humidity = 53.5 ± 5.4% and ambient pressure = 684 ± 4 mm Hg). During the NBR and HBR campaigns the participants were confined to strict horizontal bed rest, a valid ground-based model to simulate microgravity-induced metabolic and cardiovascular alterations.

During the HAMB confinement the participants were encouraged to engage in their habitual routines and performed two 30-min low-intensity exercise sessions per day to mimic their habitual physical activity. The normobaric hypoxic environment was provided using the Vacuum Pressure Swing Adsorption system (b-Cat, Tiel, The Netherlands) that delivered the O_2-depleted gas to the designated area.

The participants received an individually tailored, strictly controlled and standardized diet throughout all three phases of each campaign. Individualized caloric requirements were calculated using the modified Harris-Benedict resting metabolic rate equation [13] and subsequently multiplied by activity factors of 1.4 for the ambulatory confinement and of 1.2 for both bed rest confinements. The same daily menu was employed during all campaigns to ensure the participants consumed identical meals on the same days of each respective campaign.

2.3 Body composition assessment

Body mass was determined every morning with participants in the supine position and fasted, using a calibrated, custom-made gurney with load cells (Sigma 6C, Libela ELSI, Celje, Slovenia).

Body composition was assessed before (Pre), immediately after (Post) and 14 days following each confinement period (Rec) using a dual-energy X-ray absorptiometry (DXA; Discovery W - QDR series, Hologic, Bedford USA). The device was calibrated prior to the first scanning according to the manufacturer's instructions. To optimize the reproducibility of the DXA measures the participants were always scanned fasted and well rested. They maintained supine position on the DXA table throughout the scanning procedure. Two scans were performed at each testing period and the average of the duplicate measures was employed to estimate the whole body and regional values of fat free mass (FFM), fat mass and % fat mass. The regions of interest used for the analysis are detailed elsewhere [12].

Statistical analysis

Data are expressed as mean ± SD unless otherwise indicated. Two-factor (campaign × time) repeated-measures ANOVA was used to define the differences in the average daily body mass as well as (Pre-Post-After) changes in body composition. Tukey's HSD *post hoc* test was employed to define the specific differences when main effect or an interaction was noted. All analyses were performed using Statistica 12.0 (Statsoft, Tulsa, USA).

3 RESULTS

3.1 Diet

The average daily energy intakes were lower than targeted in all campaigns (NBR: -5%; HAMB: -14%; HBR: -6%; $P < 0.01$). The energy and macronutrient intakes were higher in the HAMB compared to NBR and HBR campaigns.

3.2 Body mass and composition

Body mass significantly decreased after all campaigns (NBR: Pre = 74.9 ± 3.3, Post = 72.9 ± 3.0; HAMB: Pre = 76.9 ± 4.3, Post = 73.7 ± 4.0; HBR: Pre = 76.1 ± 3.3, Post = 72.5 ± 3.3; $P < 0.01$; Figure 1). No differences in body mass reduction profiles were observed between the campaigns.

For whole body FFM, a main effect of time ($P < 0.01$) and a campaign × time interaction ($P < 0.01$) were noted. Whole body FFM decreased at Post following all campaigns (NBR = -4%, HAMB = -5%, HBR = -5%; $P < 0.01$) and remained reduced at Rec only following the NBR ($P < 0.01$) and HAMB ($P < 0.01$) campaigns only (Figure 2). No main effect was observed for changes in whole body fat mass ($P = 0.19$) and whole body % fat mass ($P = 0.12$) during and after the campaigns (Figure 2).

In regards to the regional composition changes a main effect of time was observed for FFM in the lower leg ($P < 0.01$), thigh ($P < 0.01$) and upper arm ($P < 0.05$). The FFM was reduced in both the lower leg ($P < 0.01$) and thigh ($P < 0.01$) at Post and returned to baseline at Rec in all three campaigns. No main effect or an interaction was observed in either region for changes in fat mass ($P = 0.08$) and % fat mass ($P = 0.16$).

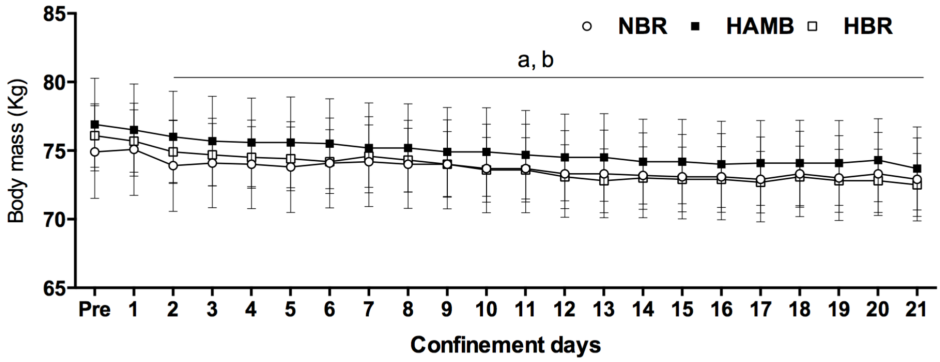

Figure 1: *Body mass profile before (Pre) and during the normoxic bed rest (NBR; open circles), hypoxic ambulatory confinement (HAMB; closed squares) and hypoxic bed rest (HBR; open squares) campaigns (Mean ± SEM). Significant effects (P < 0.05): [a] main effect time; [b] interaction (campaign × time).*

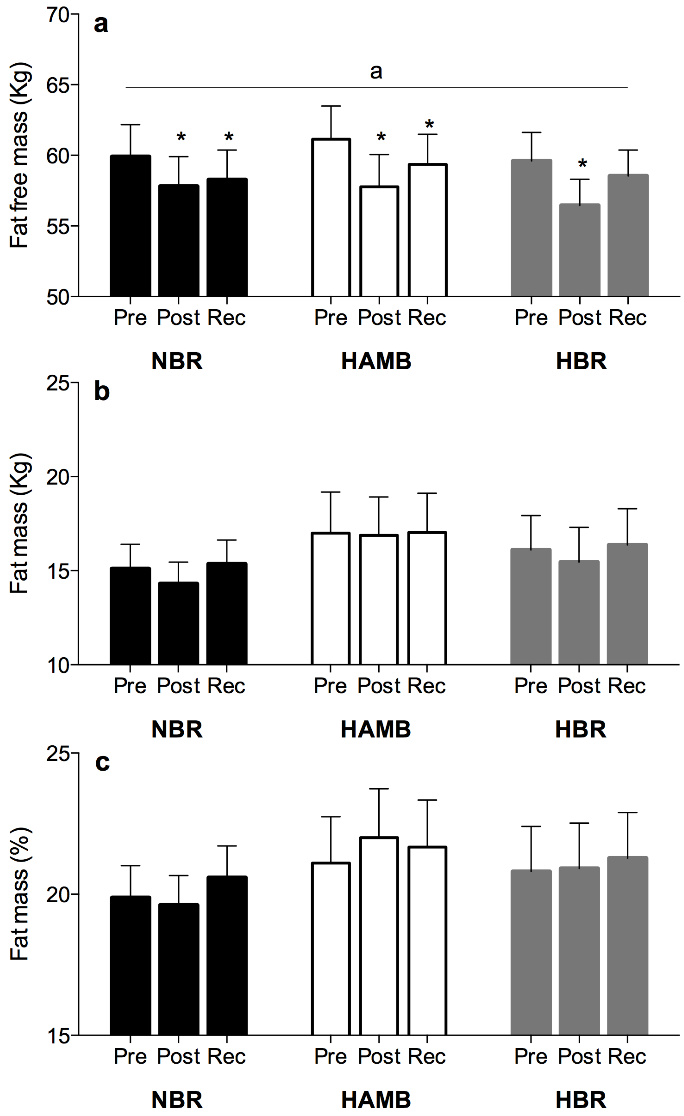

Figure 2: *DXA estimated whole body fat free mass (a), fat mass (b) and % fat mass (c) before (Pre), immediately after (Post) and 14 days after (Rec) the normoxic bed rest (NBR), hypoxic ambulatory confinement (HAMB) and hypoxic bed rest (HBR) campaigns (Mean ± SEM). Significant effects (P < 0.05): [a] main effect time. Significant post hoc differences: * (P < 0.01) vs Pre*

4 CONCLUSIONS

While both unloading [1,14] and hypoxia [4,6] can result in reduced body mass no additive effect of hypoxia was observed in the present study. Similar body mass reductions were noted following all three experimental campaigns. Furthermore, under appropriate diet, unloading mostly induces muscle volume reductions [15], whereas hypoxia affects both muscle and fat tissue [5]. However, when comparing both the whole and regional body composition results following the NBR and HBR no “hypoxia” specific effect could be identified. The only difference between the two bed rest confinements was that the FFM remained reduced at Rec only following the NBR (Fig. 4). The faster regain of the FFM following the HBR campaign might suggest the contribution of hypoxia-induced water shifts between the extracellular and intracellular compartments [16]. It is indeed surprising that the FFM reductions following the HAMB were similar to those observed during the NBR and HBR. Furthermore, significant reduction in the upper arm FFM was only observed following the HAMB. While the latter might not be of significant relevance, the HAMB did result in significant whole-body FFM reduction. The potential factors underlying these FFM reductions include insufficient energy and macronutrient intakes, water content shifts and reduced physical activity levels.

In conclusion, hypoxia did not additively reduce body weight or influence body composition modulation following 21 days of bed rest. Interestingly, reductions in body mass following hypoxic ambulatory confinement were similar to those observed after both bed rest confinements. Potential explanations for this observation include insufficient energy intakes secondary to appetite reduction, low activity levels and/or effects of confinement *per se.*

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THE ANALYSIS OF SEASONAL TRAINING OF ELITE BREATH-HOLD DIVER. A CASE STUDY

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ABSTRACT

The purpose of the present study was to ascertain whether the changes in training interventions due to seasonal training periodisation were accompanied by changes in the diver's ability to perform maximal face immersion apnea. A male, world-class apnea diver was followed for 1 year (from April 2012 to April 2013). During this period he was tested six times. Each test session involved the measurements of the pulmonary function and respiratory muscle strength. In addition, the ability to perform maximal face immersion apnea was also explored. The results of face immersion apnea durations showed a continuous improvement throughout the preparation period with the peak in the competition period and a decline during the post-competition period. It seemed that the training periodisation was successful by producing the diver's peak performance level at the main diving competition i.e. the 2012 AIDA Freediving World Championships. In conclusion, the study shows that changes in training interventions due to seasonal training periodisation could be accompanied by changes in a diver's ability to perform the maximal face-immersion apnea. However, further research is needed to establish the influences of individual components of apnea training on a diver's performance.

1 INTRODUCTION

Competitive breath-hold (apnea) diving includes several disciplines in which the diver performs at their maximum regarding depth, duration or distance. Besides depth disciplines, some apnea diving disciplines are performed in a swimming pool (e.g. static apnea and dynamic apnea with or without fins). International diving competitions usually include all or several of these disciplines, and most athletes compete in all disciplines. This makes specialisation difficult, requiring training for overall performance rather than discipline-specific training [1]. Thus, a competitive driver's training consists of a series of static dry and immersed apneas, dynamic apneas in a swimming pool and, in the weeks leading up to a competition, dynamic deep diving in a sea or lake. Apnea training is usually combined with endurance training like swimming, running and cycling, strength training and flexibility training. These various types of training are often manipulated so as to produce the peak performance level at the main diving competition.

The present paper reports the results of a 1-year study of a world-class male diver. At the time of this work, the diver was preparing for the 2012 AIDA Freediving World Championships in Nica and had undergone regular physiological testing. The purpose of the physiological monitoring was to ensure that the diver was in peak physical condition for the major event. Apart from spirometry and measurements of breathing muscle strength, it involved measurements of selected variables during maximal face-immersion apnea (FIA). Therefore, the purpose of the present study was to ascertain whether the changes in training interventions due to seasonal training periodisation were accompanied by changes in the diver's ability to perform maximal FIA.

2 METHODS

2.1 Subject

A male, world-class apnea diver, age 32 years, 90 kg (body weight), and 192 cm (height) was followed for 1 year (from April 2012 to April 2013). At the beginning of the study he had 10 years of competitive apnea diving experiences. His training consisted of a preparatory period (from April to July), a competition period (August, September and October) and a post-competition period (from November to April).

2.1 Testing protocol

The diver underwent six tests over the 1-year period. All tests took place in controlled environmental laboratory conditions (21°C, 40-60% RH, 970-980 mbar) and at the same time of day. He completed the following tests in the same order at each test session: 1) pulmonary function; 2) respiratory muscle strength; and 3) FIA.

Pulmonary function. The following variables were derived: vital capacity (VC), forced vital capacity (FVC) and forced expiratory volume in 1 s (FEV1.0). Pulmonary function measurements were made according to European Respiratory Society recommendations [2].

Respiratory muscle strength was assessed by measuring the maximal inspiratory mouth pressure (MIP) at residual volume and maximal expiratory mouth pressure (MEP) at total lung capacity in a standing position. The assessment of maximal pressures required a sharp, forceful effort maintained for a minimum of 2 s. The diver became well habituated with the procedure during two separate familiarisation sessions. He received visual feed-back on the pressure achieved during each effort by viewing the digital display on a hand-held device in order to maximise his inspiratory and expiratory effort.

FIA. The diver lay down in a prone position on a rubber pad, with his head on a rigid cover under which a container with water was placed. The water temperature was maintained between 25 and 27°C. He relaxed for about 10 min while the measuring equipment was attached and stable, resting values of HR and breathing parameters were achieved. Then, 30 s before the intended FIA, the diver lifted his head and the cover was removed. Immediately before the FIA, he was allowed to perform a maximal inspiration without previous hyperventilation or glossopharyngeal pistoning, a common breathing manoeuvre among experienced apnea divers to maximally increase their total lung capacity [3]. The FIA was started when the diver immersed his face. During the FIA, he was instructed to hold his breath for as long as possible. He had to show the moment when the involuntary breathing movement started, i.e. the physiological breaking point, with his hand as well. He wore a nose clip and was not allowed to exhale into the water during the breath-hold. The first expiration after the FIA was into a breathing mask. When the diver began to breathe into the breathing mask, the cover was put back on the container and he rested until the next FIA. Two FIAs were performed with a 5 min resting interval.

2.1 Data Acquisition and Analyses

A pneumotachograph spirometer (Vicatest P2a, Mijnhardt, Netherlands) was used to measure the resting flow-volume profiles. MIP and MEP were measured using a portable hand-held mouth pressure meter (MicroRMP, MicroMedical Ltd, Kent, UK). The measurements of both parameters were taken repeatedly until a stable baseline of each parameter was achieved. The criteria for determining MIP and MEP stability was successive efforts within 5%. The highest value recorded was included in the subsequent analysis. The air expired before and after the FIA was sampled continuously by a metabolic cart (V-MAX29, SensorMedics Corporation, Yorba Linda, USA) for a breath-by-breath determination of the end-tidal partial pressure of oxygen ($P_{ET}O_2$) and carbon dioxide ($P_{ET}CO_2$). The pneumotachograph and O_2 and CO_2 analysers were calibrated with a standard three-litre syringe and precision reference gases, respectively. Resting data of those parameters were the average from a 30 s period 1 min before the FIA started. HR and SaO_2 were recorded on the right middle finger with a computer-based data-collection system MP100 and Oxy100 module (Biopac System, Goleta, CA) during each FIA. Calibrated analogue signals were analogue-to-digital converted, recorded at 200 Hz per channel, and subsequently stored and analysed with the AcqKnowledge 3.2.6. software package (Biopac Systems). The recording of both parameters commenced 2 min prior to each FIA and finished 3 min after it.

As this is a 1-year case study, all training and test data are only presented in the form of descriptive statistics.

3 RESULTS

Analysis of the training diaries showed that the diver completed 18 ± 7 training sessions per month in the preparatory period (from April to July), 15 ± 5 training sessions per month in the competition period (August, September and October) and 13 ± 4 training sessions per month in the post-competition period (from November to April). The test results of the FIA durations and the times of the physiological breaking point are shown in Figure 1.

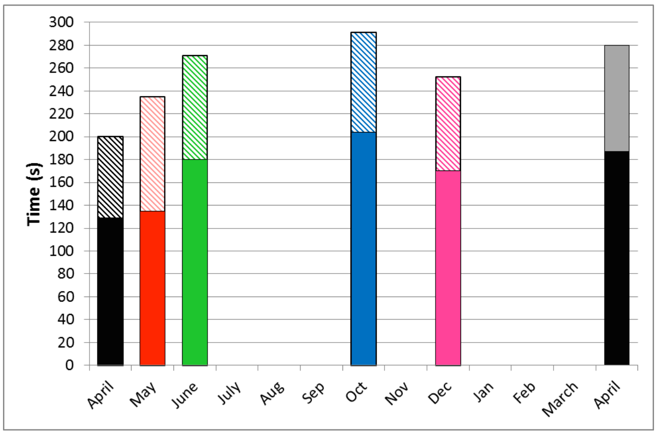

Figure 1: *The durations of the face-immersion apneas (columns with lines) and the times of the physiological breaking points (full columns) from April 2012 to April 2013.*

Figure 1 shows that the FIA was becoming longer throughout the preparation period, peaked in the competition period and was becoming shorter during the post-competition period. These results were in accordance with the training periodisation. During the preparation period, the FIA duration was extended by 35%. In addition, the physiology breaking point appeared about 39% later during the FIA in June compared to the FIA in April. At the September test session, i.e. testing during the competition period, the longest FIA and the most delayed physiological breaking point were obtained. During the post-competition period, the FIA durations were shorter than in the September testing, although still longer than during the preparation period.

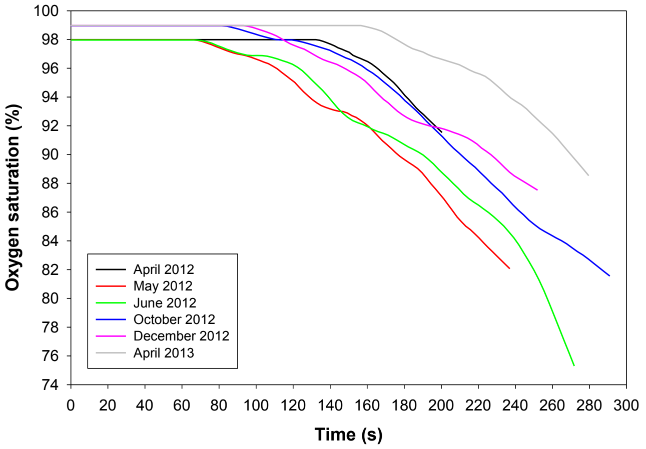

Figure 2: *Oxygen saturation curves during the face-immersion apneas.*

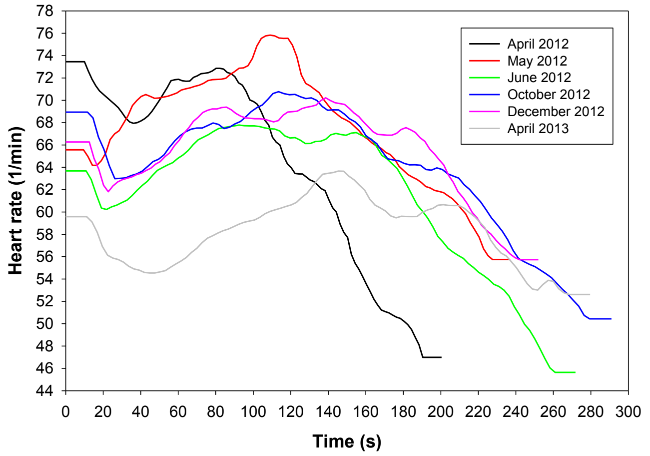

Figure 3: *Heart rate curves during the face-immersion apneas.*

Considering the SaO_2 curves during the FIA, there were differences in the duration of the initial plateau of stable values before any desaturation happened. The shorthes was in the May and June testings. On the other hand, grey curve in Figure 2 shows that the longest initial plateau was in the April 2013 testing. In addition, it seemed that the rate of oxygen desaturation did not vary between the FIAs. Interestingly, the lowest values of SaO_2 was obtained at the end of June's FIA, which was not the longest.

Figure 3 shows that after initial bradycardia, a further increase of HR occured. Close to the physiological breaking point, the values started to decrease to the minimum obtained at the end of the FIA.

	April 2012	May 2012	June 2012	October 2012	December 2012	April 2013
$P_{ET}CO_2$ before FIA (kPa)	5.2	4.9	4.3	4.8	5.2	4.7
$P_{ET}CO_2$ immediately after FIA (kPa)	7.3	7,4	7,5	7	7,3	7,1
VC (l)	8.33	8.69	8.48	8.37	8.11	8.03
VC with packing (l)	9.76	9.75	9.6	9.48	9.76	9.2
FVC (l)	8.19	8.32	7.86	8.38	7.96	7.9
FEV1 (l/s)	5.75	5.82	5.42	5.7	5.43	5.37
MIP (cmH_2O)	117	128	122	114	108	112
MEP (cmH_2O)	185	179	182	182	189	185

Note: $P_{ET}CO_2$. end-tidal partial pressure of carbon dioxide; FIA. face-immersion apnea; VC. vital capacity; FVC. force vital capacity; FEV1.0. forced expiratory volume in one second; MIP. maximal inspiratory mouth pressure; MEP. maximal expiratory mouth pressure.

Table 1: *The results of end-tidal partial pressure of carbon dioxide measured before and after the face-immersion apneas and the test results for the spirometry parameters and the parameters of respiratory muscle strength from April 2012 to April 2013.*

Table 1 shows that VC varied for about 8% measured at different testings. On the other hand, there were only little variation in MIP and MEP thus diver's inspiratory and expiratory muscle strength throughout the season. In addition, despite different FIA durations, $P_{ET}CO_2$ measured immediately after the FIA did not difer throughout the season.

3 DISCUSION

The world-class apnea diver was followed throughout the season in the current study. The main finding in this study is that changes in training interventions due to seasonal training periodisation could be accompanied by changes in a diver's ability to perform maximal FIA.

Even though apnea diving disciplines have varying key physiological features that determine individual performance, sufficient apneic duration is the ability which is crucial to all apnea sports [1]. The results of FIA durations in the present study showed a continuous improvement throughout the preparation period with the peak in the competition period and a decline during the post-competition period (Figure 1). It seemed that the training periodisation was successful by producing the diver's peak performance level at the main diving competition i.e. the 2012 AIDA Freediving World Championships. However, to our knowledge only a few longitudinal studies have been performed to clarify which components of apnea training are responsible for such an adaptation. Therefore, it could not be concluded that any particular type and/or manipulation of the various types of apnea training per se is responsible for the improvement or decline in the FIA duration during the season in the present study. The FIA durations throughout the 1-year period varied from 200 to 291 s (Figure 1). The maximal FIA duration reported using a similar protocol in members of the Croatian national apnea diving team was, on average, a minute less than in the present study [4]. Further, even the results of the previous freediving world record holder were a little lower (182–305 s) [5]. With this in mind, the current diver may be classified as elite.

Beside the apnea duration, the diver's ability was evaluated by analysing HR and SaO_2 during the FIA. In previous studies, the magnitude of bradycardia has often been used to estimate the overall magnitude of the diving response. The analysis of HR curves showed that there were three phases during the FIAs in the present study. Considering the long durations of apneas such curves were expected [6, 7, 8]. At the beginning of the FIA, HR dropped continuously, reaching the lowest values about 32 (±16)s after facial immersion. This decrease was about 23 (±2)% due to the resting values (Figures 3), which is consistent with previous studies [9, 10]. After the initial phase, HR started to increase and reached the maximum at an average 128 (±28)s after facial immersion. This increase was about 20 (±5)% due to the resting values (Figure 3). After peaking, a progressive reduction in HR occurred in the present study. The HR reduction could be explained with at least two phenomena that happened at the same time during the apnea. First, it was speculated that the start of the HR reduction might correspond to the physiological breaking point of the apnea, i.e. the point when involuntary respiratory movements begin to occur [7, 8, 11]. This speculation was confirmed in the present study. Indeed, the physiological breaking point and the beginning of the HR reduction were obtained at 54 (±12)% and 66 (±5)% of the FIA duration, respectively (Figures 1 and 3). Further, it seemed that the changes in these two variables were similar to the changes in FIA durations throughout the season. Thus, the later the physiological breaking point and the beginning of the HR reduction appeared, the longer the FIA was.

The cardiovascular responses (bradycardia, hypertension) to FIA which were partially explained above are believed to be a defensive reflex designed to reduce peripheral oxygen consumption, while maintaining oxygenation to the heart and brain [5]. The analysis of the SaO_2 curves showed that there were initial plateaus without any significant changes in SaO_2 at the beginning of the FIA. Its duration varied between 102 and 183 s during the season (Figures 2). This is in line with data from previous studies in which the initial period lasted approximately 1 min 14 s and was longer with trained free divers compared to untrained subjects [5]. The reason for the varied duration of the initial plateau of SaO_2 in the present study could be different volumes of the last inspiration before the FIA [5]. The diver standardised the manoeuvre of the last inspiration before each FIA and did not change it throughout the season. However, different last-inspiration volumes before the FIA could be possible since the diver's VC varied by about 8% during the season (Table 1). After the initial period, SaO_2 decreased to the minimum value occurring at the end of the FIA. The lowest value of SaO_2 of 75% was obtained at June's testing (Figure 2). It seemed that slope of the desaturation curves did not differ between each FIA and thus the minimum values were related to the duration of the FIA.

4 CONCLUSION

In conclusion, the study shows that changes in training interventions due to seasonal training periodisation could be accompanied by changes in a diver's ability to perform the maximal face-immersion apnea. However, further research is needed to establish the influences of individual components of apnea training on a diver's performance.

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EFFECTS OF COGNITIVE STIMULATION DURING SIMULATED MICROGRAVITY ON MOBILITY AND BRAIN ELECTROCORTICAL ACTIVITY OF OLDER ADULT MEN

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ABSTRACT

Prolonged physical inactivity or bed rest (BR) due to illness or exposure to microgravity in space flights can result in significant declines in cardiovascular, sensory-motor, musculoskeletal, or even cognitive functions. A virtual spatial navigation training paradigm was proposed as a technique to mitigate mobility-related declines following prolonged BR. The aim of this study was to evaluate the effects of such a computerized cognitive training (CCT) approach during 14-day BR separately on walking performance and electrocortical activity. Fifteen healthy older men (59.6 years old) completed a 14-day BR study. Seven were randomly chosen for the CCT program involving 12 sessions of spatial navigation training during the BR period, while eight other participants watched documentaries for a similar period of time. Before and after the BR, gait performance and electrocortical activity was measured. Results showed that participants in the Intervention group as compared to the Control group demonstrated significantly reduced negative dual-task effects (DTEs) after the BR in both the normal ($p < .001$) and fast paced walking conditions ($p = .03$). Baseline electrocortical activity measurements at post-BR as compared to pre-BR showed an increase in theta power in the Control group and an increase in Beta2 power in the Intervention group. Our findings showed that CCT using a spatial navigation intervention reduced negative DTEs in walking performance and had a significant effect on the brain electrocortical activity, most likely explained by the engagement of brain structures known to be involved in mobility or improving cognitive domains associated with control of mobility.

1 INTRODUCTION

Bed rest studies are recognized as a valid model for simulating and studying an acute stage of human adaptation to the microgravity in space flights, sedentary lifestyle and the impact of long-term postoperative immobilization [1]. It is well established that bed rest has detrimental effects on several systems of human body, including cardio-vascular, skeletal or muscular systems, with a scarce literature examining brain functioning [2]. Moreover, little is known about the effects of computerized cognitive training (CCT) incorporation spatial navigation during simulated microgravity on mobility and brain functioning domain.

Despite other brain imaging techniques (magnetic resonance imaging, positron-emission tomography, diffusion tensor imaging), electroencephalography (EEG) could be a method of choise in the extreme environments (e.g. in the microgravity condtions in space), due to its portability. Scalp EEG has been a routine research and clinical tool, even though its relationship to the underlying neural activity remains incompletely understood. Despite the small magnitude of measured scalp potentials, the surface EEG signal reflects summation of synchronized afferent or efferent, excitatory or inhibitory, neural activity. The predominant "slow wave" activity (delta and theta) is indicative of lower cortical activity and can be indicative of neuropathology and/or ageing process, while increased "fast activity" (alpha and beta) is indicative of increased arousal and cognitive engagement [3].

Longer bed rest studies investigating brain functioning in terms of EEG analysis are scarce. Two abstracts from Chinese authors reported findings of a six days head down tilt (HDT) bed rest on human EEG spectral changes in younger adult men. Alpha power increased during the bed rest, while peak frequency of EEG gradually slowed down. Beta1 and theta rhythms increased and reached maximum on the 3rd day at the frontal regions. The authors concluded that "there exists a potential influence of bed rest on brain functioning, headache can be easily induced and that brain adaptation function decrease" [4, 5].

The aim of the present study was to evaluate if CCT incorporating spatial navigation could be an effective technique for reducing/preventing negative effects of 14 days of bed rest which are reflected in gait performance and brain electrocortical activity.

2 METHODS

2.1 Participants

Sixteen men (59.6 ± 3.4 years, height 174.5 ± 4.8 cm, body mass 79.6 ± 13.0 kg, BMI 26.2 ± 4.5 kg/m2) participated in a 14-day bed rest study: Eight of them underwent 12 daily sessions of 50 minutes computerized cognitive stimulation in which participants had to solve virtual mazes, while the Control group (n = 8) watched documentaries in the separate room for the same amount of time. To achieve the aim of the study and to simulate physical inactivity (simulated microgravity conditions), all participants had to rest in bed for 14 days. During the bed rest, the participants were only allowed to turn on all sides of the body or put no more than two pillows under the head and were not allowed to stand up, sit on the bed, or raise their arms above the level of their head. All participants were right-handed, had normal or corrected-to-normal vision, and reported no history of cardiovascular, neurological, or psychiatric conditions. All procedures were carried out in accordance with the Declaration of Helsinki and were approved by the National Medical Ethics Committee. Written informed consent was obtained from all participants prior to the study.

2.2 Cognitive stimulation

The Intervention group performed 50 minutes of computerized cognitive training with spatial navigation intervention each day from the 2nd to the 13th day of the study. During the periods of training, all the participants were lying in a supine position and moved through the virtual environment using a joystick device (Trust Predator Joystick GM-2550). The tasks were presented on a 17-inch flat panel LCD monitor situated at a distance of approximately 60 centimeters in front of each participant. The training program consisted of virtual mazes representing a series of interconnected corridors, with three available paths at each intersection or decision-making point. For each maze, either a pair of verbal or pictorial cues were displayed at each decision-making point, placed at either opposite corner of the intersection and in corridors at various non-decision-making points. Verbal cues consisted of signs with country names, city names, and animal names. After being familiarized with the joystick and navigation in the virtual maze environment, participants were instructed to select the correct path as quickly and efficiently as possible in order to move toward the goal area. The training was performed using several virtual maze environments, each of increasing difficulty, designed using a modified version of Unreal Tournament 2003 and the Unreal Editor 3.0 (Epic Games, Inc.) software package [6].

2.3 Mobility measurements

Spatio-temporal gait parameters were measured with Optogait system (Microgate, Bolzano, Italy). Data was sampled at 1000 Hz and analyzed with OptoGait software, version 1.6.0. Participants were asked to start walking with the right foot on each starting cycle and continue walking for 1 minute. Participants walked in their preferable self-selected speed, brisk walking to the best of their capacity, and both speeds under a dual-task condition in a randomized order (see Figure 1, left panel). The dual-task condition was walking and at the same time subtracting by threes from a randomly chosen number between 400 and 500. Each participant completed one minute of walking for each testing conditions, starting out always with the right foot.

To be able to compare single- and dual-task conditions, we applied the dual-task effect (DTE) model [7]. DTEs are a sensitive relative measure allowing comparison of single- and dual-task walking conditions. The measure of DTE was calculated as follows:

$$DTE = \frac{\text{dual task walking condition} - \text{single task walking condition}}{\text{single task walking condition}} \times 100$$

A negative values of DTE represent a decrement called dual-task costs, while a positive values represent an improvement under dual-task condition, called dual-task benefits. Dual-task costs reflect the increased cost of cognitive attentional processes under the dual-task condition [8], while dual-task benefits may represent higher arousal under more challenging dual-task condition or that secondary task focused attention away from walking [9]. Gait variability was assessed with swing time variability of the gait cycle [10].

2.4 Electroencephalographic measurements (EEG)

A day before and after bed rest we recorded baseline EEG with active 64 channels system in the eyes-open and closed conditions. Baseline EEG was decomposed with Fast Fourier Transform (FFT) to 7 different band ranges:

- theta (4–7.5 Hz),
- alpha 1 (8–10.5 Hz),
- alpha 2 (11–12.5 Hz),
- beta 1 (13–19.5 Hz), and
- beta 2 (20–29.5 Hz).

Prior to FFT, EEG epochs were transformed into the reference-free current source density (CSD) distribution. 64 channels were pooled in 6 regions of interest (ROI): frontal, left and right temporal, central, parietal and occipital (see Figure, middle panel).

The EEG data was analyzed with Brain Vision Analyzer 2.0 (Brain Products GmbH, Germany).

2.5 Statistical analysis

Statistical analysis was performed with IBM SPSS Statistics 20.0 software for Windows (SPSS, Inc., Chicago, Ill, USA). Normality of the distribution of the parameters was tested with the Shapiro-Wilk's test. Gait speed, DTEs, and brain electrocortical rhythms were entered into a 2 x 2 mixed design repeated measures analyses of variance (ANOVA) with Group (Intervention and Control group) as the between subject variable and Time (pre and post) as the within subject variable. Post hoc comparisons were carried out with the Least Squares Means approach. Statistical significance was set at the level of $p < .05$.

3 RESULTS

3.1 Mobility measurements

At the baseline condition no significant differences were found between both groups ($p>0.05$).
Two 2x2 ANOVAs showed no significant interaction, group or time effect in normal (single- and dual-task) and fast paced walking condition while evaluating gait speed ($p>0.050$). However, in the most demanding task (fast paced walking in dual-task condition) there was a significant time effect ($p = 0.020$, $\eta^2 = 0.350$). Post hoc analysis showed that in the Intervention group there was no significant change ($p = 0.370$), while in the Control group participants significantly reduced their gait speed ($p = 0.005$) as compared Pre- to Post- bed rest.

While evaluating DTE we found a significant interaction effect in both normal ($p < 0.001$, $\eta^2 = 0.820$) and fast-paced ($p = 0.030$, $\eta^2 = 0.310$) condition. For the normal pace walking condition participants in the Control group showed no significant change ($p = 0.124$), while participants in the Intervention group significantly reduced negative DTE (resulting as dual-task benefits) in Post- as compared with Pre-BR ($p < 0.001$). For the fast pace walking condition participants in the Control group again showed no significant change ($p = 0.716$), while participants in the Intervention group showed in Post- as compared to Pre- bed rest suggestive but not significantly smaller negative DTE ($p = 0.056$).

3.2 Gait variability
No significant changes between both groups were found at the Pre-BR baseline measurement ($p > .114$) at all eight entered parameters (four walking conditions X swing time and stride length variability).

Standard deviations of swing times were entered into 2x2 ANOVA. There were no significant interaction effects ($p > .05$) in all three walking conditions, except for the most demanding one: For fast walking with dual-task there was a trend of time [$F(1,13) = 4.29$, $p = .059$, $\eta^2 = .25$], and significant interaction effect between time and group [$F(1,13) = 12.81$, $p = .003$, $\eta^2 = .50$]. Post-hoc analysis showed that the significant effect of time was driven by Control group: while there was no significant change in the swing time variability between Pre- and Post-BR for Intervention group ($.017 \pm .005$ vs $.015 \pm .003$ seconds, $p = .302$), participants in the Control group showed significantly higher swing time variability in Post- as compared with Pre-BR (Pre-BR $.013 \pm .005$; Post-BR $.021 \pm .009$ seconds, $p = .006$).

For stride length variability, no significant interaction or time effects were found. However, the 2x2 ANOVA showed a significant group effect in fast walking with dual task ($p = .018$, $\eta^2 = .36$). Post hoc analysis showed that there were no differences at the Pre-BR measurement between the two groups ($p = .125$), while there was a significant difference at Post-BR ($p = .034$). The Control group had higher stride length variability at the end of the BR (Pre-BR 5.88 ± 1.25; Post BR 6.25 ± 1.58 cm), compared to the Intervention group, for whom stride length variability stayed at the same mean level (Pre-BR 4.71 ± 1.50; Post BR $4.71 \pm .76$ cm).

3.3 EEG measurements
In the eyes-open condition the power of theta did not change for older adult men who underwent cognitive training, while for the controls there was significant increase on theta power over posterior cortices (Figure 1, right panel).
In the eyes-closed condition there was a significant effect of bed rest and computerized cognitive training in Theta and Beta 2 frequency ranges. For theta oscillations there was a significant interaction at central ($p = .040$), parietal ($p = .030$) and occipital ($p = .004$) ROIs (2x2 group, time ANOVA), a significant decrease in the central ROI in the Intervention group ($p = .018$), and significant increase in the occipital ROI ($p = .016$) in the Control group. In the beta 2 range ANOVA showed significant interaction in frontal ($p = .048$), right ($p = .029$) and left ($p = .047$) temporal and occipital ($p = .023$) ROIs. These interactions were driven by a significant increase in Beta 2 power in the Intervention group in the frontal ($p = .006$) and right temporal ($p = .012$) ROIs.

4 CONCLUSIONS

The present study aimed to investigate the influence of computerized cognitive training with virtual spatial navigation task on gait performance and brain electrocortical activity after a 14-day bed rest in older adult men. To the best of our knowledge, this is the first study to apply a cognitive training intervention during prolonged physical inactivity in highly controlled environment.

Participants in the Intervention group as compared to controls showed no significant reduction in gait speed after bed rest and a significant improvement in DTEs (a switch from dual-task costs to benefits) in gait speed with normal pace walking. In the fast pace walking condition, the post-intervention reduction of negative DTEs was marginally nonsignificant ($p = .056$). Participants in the Control group, however, showed approximately the same level of negative DTEs in Pre- as compared with Post-BR, in single-task as well as in dual-task conditions. The Intervention group also did not demonstrate the same reduction of gait speed as did the controls in the fast paced dual-task walking condition after the BR. Furthermore, gait variability in the Control group showed significantly higher swing time variability at the end of BR in the most demanding walking condition (fast dual-task walking condition) as compared to the Intervention group. Thus, the results from our study suggest that cognitive stimulation can significantly reduced the detrimental effects of 14 days bed rest, measured on motor output and the brain electrocortical activity.

Most studies which applied cognitive training (in a non-bed rest setups) revealed effectiveness of cognitive in the same/

trained domain, showing only limited transfer [11, 12]. However, Verghese and collegues showed a transfer of computerized cognitive training on mobility domain in sedentary seniors [13]. Same authors reported that cognitive stimulation (targeting attention and executive functioning) could have translated into more efficient walking pattern during most demanding dual-task walking condition [13].
While analyzing results from the brain electrocortical acitivity, results from our study showed that bed rest "slowed" EEG (increase in theta power) in the Control group, while this was reverse in the Intervention group (increased power in beta 2 range). The "slowing" of EEG caused by bed rest could reflect diminished cognitive functioning while "speed-up" is indicative of greater alertness and attention in our Intervention group. Furthermore, an increase in theta power is characteristic in subjects with variety neurological disorders and for the aging process (late part of the life span) (Klimesch, 1998).
It has been well established that success in spatial navigation is associated with superior spatial memory, speed of processing, as well as executive functions [14], and that during regular walking and especially in dual-task walking, similar cognitive processes are engaged [15]. Thus, CCT may have been most successful during the dual-task walking condition because efficient dual-task walking requires attention, appropriate speed of processing, and executive functions that are trained by the CCT [13]. Virtual spatial navigation also has been shown to activate neural structures involved in mobility [14, 16]. Lovden and colleagues [16] recently reported that sustained spatial navigation training mitigates age-related declines of hippocampal volume, a critical brain structure for long and short term memory and one closely involved in spatial navigation.
Overall, our results suggest that bed rest has detrimental effects on the brain electrocortical activity while CCT intervention could prevent this deleterious effect. Future analyses will investigate potential significant associations between behavioural and brain acitivy domains. Outcomes of this study could serve to develop new intervention programs, as well as rehabilitation protocols, to be used in situations of long term inactivity caused by illness, injuries, or space flights.

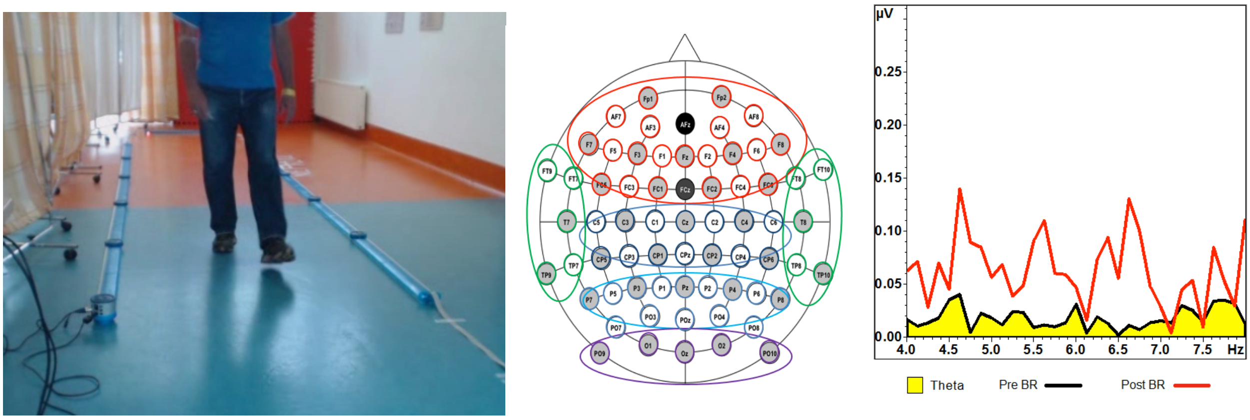

Figure 1: Left panel: *Example of gait measurement with Optogait system; Middle panel: Regions of Interest (ROI): frontal (red), Left and Right temporal (green), central (dark blue), parietal (light blue), and occipital (purple) in EEG analysis; Right panel: Significant increase of theta band after the BR in the control group at central ROI.*

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PERIPHERAL PERFUSION AND ACUTE MOUNTAIN SICKNESS: IS THERE A LINK?

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BACKGROUND Overall peripheral perfusion during hypoxic exposure is a result of interplay between vasodilatation and sympathetic vasoconstriction. Loeppky et al. (2003), suggest that acute mountain sickness, body temperature and autonomic responses are directly related. Previously, we investigated the circadian rhythm of peripheral perfusion reflected in the proximal to distal skin temperature gradient (ΔTp-d) in bedrest and simulated altitude (McDonnell et al. 2014). Anecdotally, many of our participants subject to chronic hypoxia and confinement tended to complain regarding the negative effects of hypoxia in the afternoon and early evening. Therefore, the main aim of the present study was a retrospective analysis of 3 separate studies investigating the circadian rhythm of healthy males confined to simulated altitude.

METHODOLOGY Thirty-six males in total participated in 3 separate studies as follows: LunHab, 11 participants (10-day hypoxic bedrest and confinement), PlanHab, 11 participants (21-day hypoxic bedrest and confinement), HECS, 14 participants (10-day hypoxic confinement). Twenty hours of continuous measurement of the proximal to distal skin temperature gradient (calf and toe respectively, ΔTc-t) were recorded on the first day of simulated altitude (~4000m). The participants completed the Lake Louise Mountain Sickness question in the evening of the first day between 20:00 and 21:00. Distal and proximal skin temperatures were recorded at 1-minute intervals and then averaged into 60-minute epochs. The ΔTc-t data was collated into two groups, those who presented with acute mountain sickness (AMS) and those who did not (nAMS).

RESULTS Sixteen participants presented with AMS on D1 of the hypoxic exposure. The ΔTc-t decreased in the AMS group at 16:00. A significant difference in the ΔTc-t gradient between the AMS and nAMS group persisted until 19:00 on D1 of hypoxic exposure. At 21:00 the difference in ΔTc-t between the two groups had abated, whereby both groups showed significant vasodilatation of the toes thereafter. There were no differences between groups in terms of age, body mass, body fat % or Vo_2.

CONCLUSIONS The main finding of the current study is that hypoxia-induced daytime vasoconstriction is more pronounced in those suffering from AMS during the evening hours (16:00 to 19:00). This increased hypoxia-induced vasoconstriction in individuals with AMS suggests that they may be at a greater risk of cold injury when exposed to extreme cold at high altitude.

ACKNOWLEDGEMENTS These studies were funded by the European union programme FP7 (PlanHab project; Grant no. 284438), the European Space Agency (ESA) Programme for European Cooperating States (ESTEC/Contract No. 40001043721/11 / NL / KML: Planetary Habitat Simulation), and the Slovene Research Agency (Contract no. L3-3654: Zero and reduced gravity simulation: The effect on the cardiovascular and musculoskeletal systems).

TENSIOMYOGRAPHY AMPLITUDE – A POTENTIAL NON-INVASIVE SKELETAL MUSCLE PRE-FRAILTY PARAMETER

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ABSTRACT

The aim of the study was to identify the time dynamics of muscle tone and atrophy changes when submitting 10 healthy males (24.3 ± 2.6 years) to a 35-day 7° head-down tilt bed rest followed by a 30-day recovery. Tensiomyography (TMG) amplitude (Dm) was used to assess the tone and atrophy of vastus medialis obliques (VMO) and biceps femoris (BF) and ultrasonography was used to measure their thickness. Assessments were performed on a daily basis for the first ten days of bed rest, and then on the 16th, 28th and 35th day, and also on the 1st, 3rd and 30th day of recovery. We found that TMG Dm increased rapidly already after the first day of bed rest and continued to rise until the last day, while thickness decreased after the 7th or even the 16th day of bed rest in VMO and BF, respectively. Furthermore, while the TMG contraction time (Tc) did not change in VMO; it did, however, permanently increase in BF without reversing during recovery. It can thus be concluded that TMG amplitude is much more sensitive to early adaptations towards muscle atrophy, while the TMG Tc demonstrated clinically important information about the irreversibility of muscle contractile parameters after bed rest. Both these main findings might have an important application in preventive health care, especially in assessing pre-frailty indicators.

1 INTRODUCTION

Frailty is a common clinical syndrome in older adults that carries an increased risk of poor health outcomes [1-3]. Frailty is therefore theoretically defined as a clinically recognizable state of increased health risk resulting from aging-associated decline in reserve and function across multiple physiologic systems such that the ability to cope with everyday or acute stressors is comprised. Frailty has been operationally defined [2] as meeting three out of five phenotypic criteria: low grip strength, low energy, slowed waking speed, low physical activity, and/or unintentional weight loss. A pre-frail stage, in which one or two criteria are present, identifies a subset at high risk of progressing to frailty [2].

Skeletal muscle mass is directly and indirectly included in the phenotypic criteria of frailty syndrome; however, it is also an important factor in other severe health outcomes, such as the metabolic syndrome, cardio-vascular diseases, sarcopaenia and cachexia-related diseases. Due to aging or disuse, skeletal muscle mass reduces because of a reduction in the number of motor units and atrophy of muscle fibers. The loss of muscle mass is clinically important because it leads to diminished strength and exercise capacity which presents a secondary loop targeting our health risk.

Biomedical imaging and bioimpedance methods are regularly used in clinical practice to assess muscle volume; however, due to several difficulties they are not used very frequently and neither are they used to assess early phases of skeletal muscle decline. Recently [4], the importance of such early detection has been established and an irreversible atrophy of muscle fibers has been shown to occur in older adults during recovery after a 14-day bed rest.

Tensiomyography (TMG) was first introduced in 1997 [5] and was classified as a non-invasive mechanomyographic method to assess transversal skeletal muscle belly enlargement due to lateral vibrations and thickening of the muscle during an electrically evoked twitch contraction. From TMG-based contraction time (Tc) an inter seven skeletal muscle correlation was observed between the average Tc and type 1 muscle fibre percentage [6]. However, in 2011 [7], an intra muscular correlation was observed between Tc and myosin heavy chain 1. Moreover, in [7] a more powerful multivariate correlation was observed by correlating Tc, delay and half-relaxation times to myosin heavy chain 1. Although TMG-derived time parameters have been widely explored, little is known about the TMG twitch amplitude (Dm). Only one study [8] has found a correlation between Dm increase and muscle diameter decrease after a 35-day bed rest.

Since TMG is simple to apply on every superficial skeletal muscle, we aimed at assessing the early processes of two skeletal muscles adaptations to a 35-day bed rest in parallel with an ultrasound muscle thickness assessment.

2 METHODS

Participants: Ten healthy males (24.3 ± 2.6 years) with no history of neuromuscular and cardiovascular disorders were recruited for the study. All participants were fully informed about the study procedures and the possible risks that might occur during and after the study. Prior to the study, written informed consent was obtained from all participants. The study was approved by the Republic of Slovenia National Medical Ethics Committee. All procedures with participants were in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

Study design: Participants were submitted to 35 days of 7° head-down tilt bed rest followed by 30 days of recovery. The bed rest phase was medical supervised while only the first three days of the recovery phase were supervised by kinesiologists. TMG and ultrasound assessments were applied at baseline (BDC) during bed rest (BRi, with i representing the day of bed rest) on days: 1, 2,…, 9, 10, 16, 28 and 35; and during recovery (RECi, with i representing the day of recovery) on days: 1, 3 and 30. We monitored the postural vastus medialis obliques muscle (VMO) and the non-postural biceps femoris muscle (BF) from a non-dominant site of the lower limb. TMG and ultrasound assessments were always acquired from the same anatomical points (using permanent skin markers): at the midpoint of the line above the patella and the VMO innervation point, which was detected using monophasic tetanic stimulation (impulse width 0.1 ms; frequency 10 Hz); and at the midpoint of the line between the fibula head and the ischial tuberosity on the BF. For each parameter, an average of three consequent measurements were taken for further analysis.

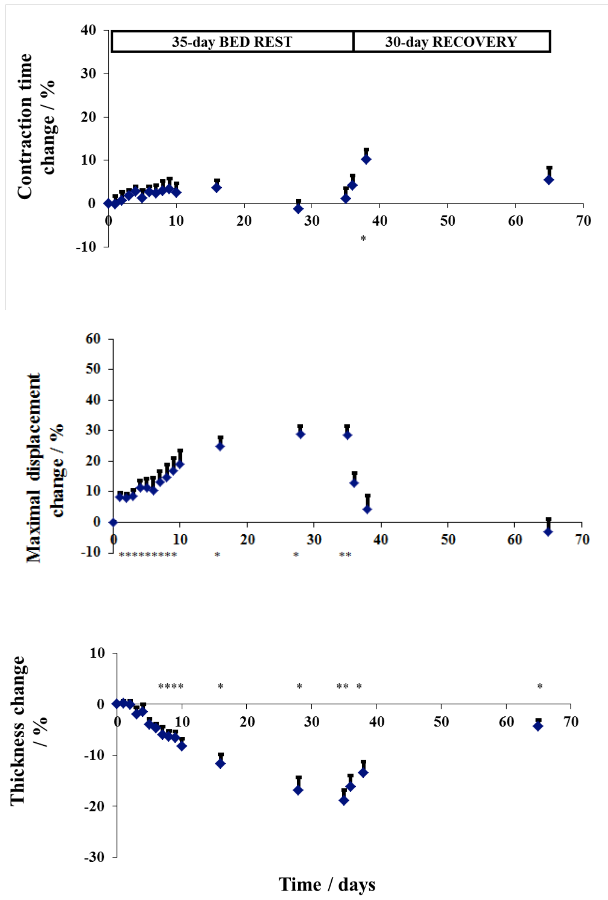
Tensiomyographic assessment: Tc and Dm were calculated as proposed by [1]: Dm was defined as a maximal displacement of muscle belly during contraction, whereas Tc was calculated as the time between 10 % to 90 % of the Dm achieved during muscle contraction. Isometric twitch contraction was triggered by supramaximal rectangular impulse (width 1 ms) through two bipolar electrodes placed 5 cm distally and proximally from the measuring point. Measurements were performed isometrically in relaxed pre-defined positions with fixed joint angles at a 5-degree knee flexion when measuring BF and a 30-degree knee flexion when measuring VMO, where 0 degrees is full joint extension.

Ultrasound assessment: Muscle thickness was acquired with ultrasonography (Esaote Mylab 25). To ensure that all subsequent scanning measurements were taken in the same anatomical plane, the ultrasound probe was positioned in the mid-sagittal plane, orthogonally to the mediolateral axis.

Statistics: All data were normally distributed. Tc, Dm and thickness data are presented with the mean and standard error. A 2-way RM ANOVA was used to check the BR/REC effect as a within factor, and muscle as a between factor. A Bonferroni correction was used for multiple comparisons when performing post hoc analyses. Significance was considered at P < 0.05.

3 RESULTS

Figures 1 and 2 present the results for all three parameters estimated from both muscles:



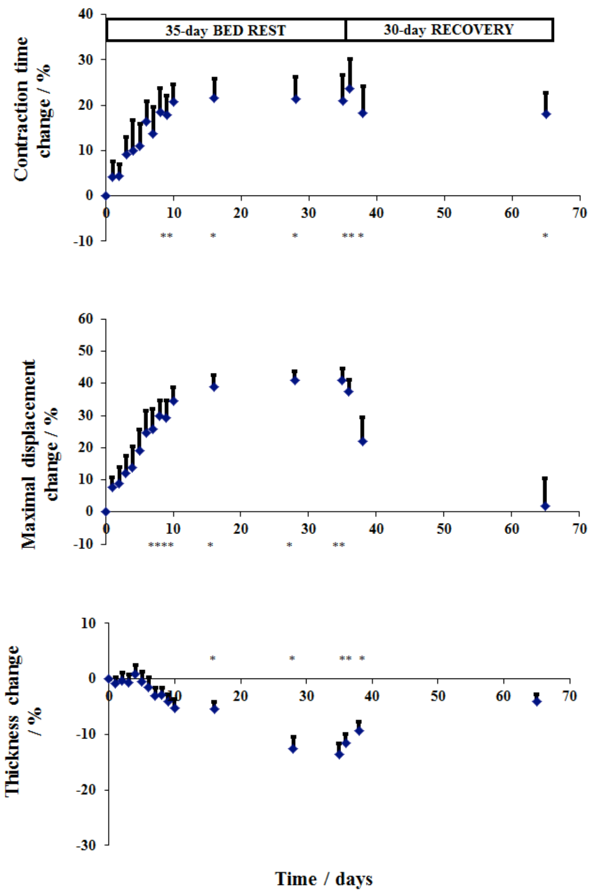
** different from baseline at P < .05*

Figure 1: *Vastus medialis obliques tensiomyographic contraction time (above) and maximal amplitude (middle), and thickness (below) change.*

Contraction time: We found a significant muscle effect (P = .010; η^2 = .314) on Tc, where the Tc of BF was 24.9% longer than that of VMO. The time effect was also significant (P < .001; η^2 = .218) where post hoc tests confirmed a significant increase in VMO Tc on REC3; however, in BF Tc a significant increase was consistent after BR9. Interaction effect was found significant at P < .001 (η^2 = .152), indicating a limited Tc change in VMO and a sustained Tc increase in BF also on REC30.

Maximal displacement: We could not confirm a significant muscle effect (P = .127) on Dm; however, the time effect was significant (P < .001; η^2 = .561), where post hoc tests confirmed a significant Dm increase in VMO and BF from BR1 and BR7, respectively. Dm was significantly increased until REC3. Interaction effect was found significant at P = .008 (η^2 = .105) indicating higher Dm changes in BF.

Thickness: We could not confirm a significant muscle effect (P = .236) on thickness; however, the time effect was significant (P < .001; η^2 = .659) where post hoc tests confirmed a significant thickness decrease in VMO and BF on BR7 and BR16, respectively. Interestingly, the thickness in VMO did not recover until REC30, but it did so in BF. The interaction effect was not significant at P = .102.



** different from baseline at P < .05*

Figure 2: *Biceps femoris tensiomyographic contraction time (above) and maximal amplitude (middle), and thickness (below) change.*

Figure 3 presents a correlation between Dm and thickness change, from which it is evident that Dm changes at higher amplitudes and also at higher initial rates than thickness in both observed muscles.

4 DISCUSSION

We aimed at assessing early phases of muscle atrophy using TMG-based parameters, where the focus was on Dm. We assumed that muscle thickness (measured as a diameter) represents the anatomical measure of muscle mass. We used thickness to estimate the decrease in muscle CSA after a 35-day bed rest, which is approx. 28% and 22% in VMO and BF, respectively, and is as such slightly higher than that observed in other disuse studies [9, 10]. Furthermore, our results indicate a higher thickness loss in postural than in non-postural muscles, which is in agreement with the results of other studies [9], indicating a greater susceptibility to atrophy of postural muscles compared to non-postural muscles. This is related to the degree of loading which muscles undergo during normal daily activity.

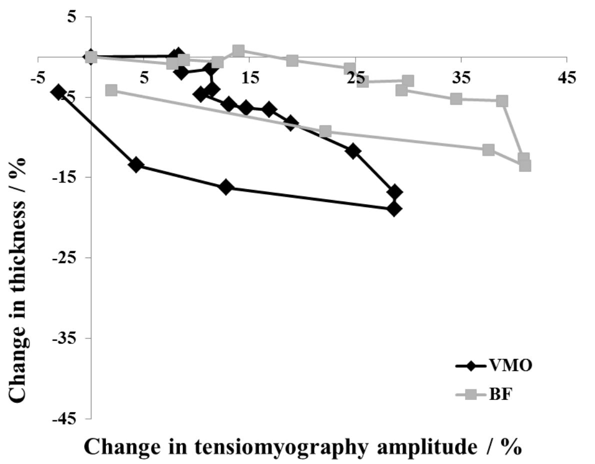


Figure 3: *A correlation presenting different dynamics in thickness and tensiomyogram amplitude change for vastus medialis obliques (VMO) and biceps femoris (BF).*

TMG is one of the mechanomyographic methods for the assessment of the displacement or oscillations of muscle belly during contractions using different techniques: phonomyographic or soundmyographic methods use microphones [11, 12]; vibromyographic methods use accelerometers and laser beams [13]; and TMG uses a displacement sensor [1]. It has already been established [8] that the amplitude of TMG (Dm) twitch response increases with the loss of muscle belly thickness; however, it had not been clear whether or not the dynamics of those changes were similar. Our results indicate different time dynamics in Dm with two clearly distinguishable phases: (i) the first phase of rapid Dm increase already on BR1; and (ii) the second phase of rapid Dm increase approx. after BR5-BR7.
The first phase could be explained by an immediate response of human body when moving from an upright to a horizontal posture with a decrease in leg and an increase in trunk muscle size [14, 15]. Fluid movement from vasculature to interstitium is likely the cause of such muscle volume alterations. This phenomenon is known as a redistribution of the body fluids or fluid shift as a consequence of gravity-dependent alterations in hydrostatic pressure.
The second phase is most probably due to a imbalance in muscle protein synthesis and degradation. Previous studies have documented that skeletal muscle mass and strength are reduced with as little as 7 days of spaceflight [16, 17] and continue to decline with the length of exposure [18]. However, there is no anatomical data available on muscle atrophy rate or function loss within the first seven days of physical inactivity exposure, although short-term physical inactivity exposures are much more frequent than the long ones.
We found that Dm clearly detects early phases of muscle adaptation to physical inactivity leading towards anatomical

atrophic changes that could be detected by traditional biomedical imaging tools. It seems that Dm reflects the loss of muscle tone change and/or fluid redistribution and as such detects predecessor processes leading towards muscle atrophy.

Furthermore, Tc gave us additional information about the muscle composition adaptation, as we were able to conclude that Tc increases in BF, but not in VMO (except on BR3, probably due to delayed muscle soreness). There is no data available on muscle composition measures for BF, while there is plenty for postural vastus lateralis or gastrocnemius medialis muscles. Interestingly, we found irreversible changes in BF Tc, which had not yet been reported and which have a high clinical relevance for redesigning rehabilitation protocols after periods of physical inactivity.

5 CONCLUSION

We can conclude that TMG gives new information on muscle adaptation in a non-invasive and simple way. Dm is clearly more sensitive to physical inactivity during the first days and also during the first days of reambulation. Furthermore, Tc detects muscle composition and we are the first to report irreversible changes for the BF muscle. Again, simplicity, non-invasiveness and selectiveness give TMG a high clinical relevance in tracking muscle adaptation to physical (in)activity.

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HUMAN MICROFLORA: A PROTOCOL FOR ASSESSMENT OF STRUCTURAL DIFFERENCES IN ESSENTIAL MICROBIAL COMMUNITIES

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Background Studies of the human microbiome have revealed that even healthy individuals differ remarkably in the microbes that occupy various body habitat types. Much of this diversity remains unexplained, although diet, environment, host genetics and early microbial exposure have all been implicated. The diversity and abundance of each body habitat's microbiological signature was shown to vary widely even among healthy subjects, with strong niche specialization both within and among individuals. We explored the use of two widely accepted approaches to population analyses, i.e. random population sampling at single time points vs. establishment of prescreened test subpopulation as a source of multiple time point samples, in order to limit the extent of random variation.

Methods The existing PlanHab project (Planetary Habitat Simulation FP7-SPACE project; http://www.planhab.com/; PI: Igor Mekjavic, IJS) was initially designed to investigate physical inactivity and hypoxia induced changes in human physiological systems for life in space planetary habitats and in specific clinical populations. This experimental setting was explored and taken up as an opportunity to obtain samples in a defined experimental setting through time from well characterized, medically monitored, and healthy subset of the general population. Protocols for solid state and liquid state NMR characterization of organic matter were established. Samples were prepared for high-throughput next-generation sequencing pipelines set up for microbiological characterization of metagenomes, microbial community structure and key microbial functional gene related to human well being.

Results The rigorous physical, medical, psychological pretesting and monitoring according to SOP for Bedrest Core Data collection (ESA, NASA) and its coupling to established analytical procedures showed great potential for analyses of human body related microbial communities and their development through time in response to experimental parameters.

Conclusion The coupling of well controlled experiments with prescreened young and healthy population of test subjects and standard operating procedures guidance in one field of research, with approaches from next generation sequencing and chemical analytics from two other fields enables noninvasive sampling of consecutive samples from all human body surface habitats (skin) and contens (feces, sputum, saliva).

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PLANHAB: INSIGHTS INTO HUMAN INTESTINAL MICROFLORA DYNAMICS OF NORMOXIC AND HYPOXIC BEDREST STUDIES

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Background The existing PlanHab project (Planetary Habitat Simulation FP7-SPACE project; http://www.planhab.com/; PI: Igor Mekjavic, IJS) that was initially designed to investigate physical inactivity and hypoxia induced changes in human physiological systems for life in space planetary habitats and in specific clinical populations, was adopted as source of human intestinal tract microbiome samples in order to study the response of healthy male subjects to three different settings: (i) normoxic bedrest, (ii) hypoxic bedrest and (iii) hypoxic ambulatory experimental variants.
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Methods Samples collected during run-in period (days -5 and -1 before the onset of experiments) and days 3, 10,18 and 21 of the three experimental settings ((i) normoxic bedrest, (ii) hypoxic bedrest and (iii) hypoxic ambulatory confination) were selected for analysis of bacterial microbial community structure. Initially fast fingerprinting approach was used to assess sample similarities and branching patterns. Deep sequencing using paired-end MiSeq approach was used for fine scale characterization and identification of key microbial groups responding to environmental perturbation. Existing human physiological data were compiled for the same days of experiments.

Results The analyses of fast fingerprinting approaches and deep sequencing showed that changes in microbial communities occurred in response to hypoxia and bedrest. The extent of change in microbial communities was not linear. Sequences were taxonomically affiliated and analyzed at 97% sequence identity to identify the most responsive groups.

Conclusion Differences in intestinal microbial communities between test subjects observed at the beginning of experiments increased through time in response to test environment and were additionally exacerbated by prolonged hypoxia. The results suggest that microbial communities respond to changes in human physiology that was changed by environmental setting.

Acknowledgements
The sample collection phase was funded by the European union programme FP7 (PlanHab project; Grant no. 284438), the European Space Agency (ESA) Programme for European Cooperating States (ESTEC/Contract No. 40001043721/11 / NL / KML: Planetary Habitat Simulation), and the Slovene Research Agency (Contract no. L3-3654: Zero and reduced gravity simulation: The effect on the cardiovascular and musculoskeletal systems). The analyses of microbial communities were financed partially through Nutrition and microbial ecology of gastrointestinal tract (P4-0097).

MUSCLE OXYGENATION DIFFERENCES RESULTING FROM LIVING AT A MODERATE OR HIGH ALTITUDE AND TRAINING AT A MODERATE ALTITUDE IN ALREADY TRAINED XC SKI-RUNNERS

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ABSTRACT

The aim of the study was to ascertain possible differences in adaptations during equal training at a moderate altitude (1500 m) but with different living conditions: at 1500 m (LMTM) or at 1500 m with additional normobaric hypoxia (FiO_2=0.18) (LHTM). A training period of 3 weeks increased the velocity of XC-ski roller running a week after LMTM from 3.17 ± 0.30 to 3.44 ± 0.33 m/s (P = 0.04), but not after the LHTM method (from 3.18 ± 0.30 to 3.35 ± 0.35 m/s; NS). There was no change in any of the parameters observed during maximal exercise and the resting haematological parameters also did not change. During similar absolute submaximal testing, LMTM maintained the concentration of oxygenated haemoglobin (O_2Hb), increased the tissue oxygenated index – TOI from -11.5 ± 65 to 1.0 ± 8.5% (P=0.03), decreased the total haemoglobin concentration (TOTHb) from 13.8 ± 7.0 to 1.7 ± 12.9 µmol (P = 0.02) and deoxygenated the haemoglobin concentration (HHb) from 24.8 ± 10.1 to 10.6 ± 12.1 µmol (P = 0.02). LHTM differently maintained muscle oxygenation with no change in TOTHb and HHb. Only the LMTM method increased oxygenation of the muscles of exercising legs and the endurance performance at a low altitude.

1 INTRODUCTION

The altitude training method live-high and train-low (LHTL) uses two specific conditions: living for at least 14 h/day at a high altitude (2400-3200 m) or in normobaric hypoxia (FiO_2 = 0.16-0.13) and training at a low, up to 1200 m terrestrial altitude [1, 2]. Training has frequently resulted in an increase in endurance, aerobic power ($\dot{V}o_{2max}$) and is accompanied by enhanced haematological characteristics [1, 3, 4]. It is believed that these alterations increase endurance in sea-level normoxic conditions through the enhancement of $\dot{V}o_{2max}$ [1, 5]. LHTL may also enhance endurance independently of haematological adaptations by increasing submaximal cycling efficiency, running economy and/or muscle buffer capacity [6]. In contrast to the presented positive effects, no influence on endurance performance and/or $\dot{V}o_{2max}$ has been found [2,4]. Finally, possible negative effects may also result from use of the LHTL method [2,7].

An alternative method, live-low and train-high (LLTH), uses two weekly, 20-40 min and moderately intense training sessions in hypoxia (FiO_2= 14.5) in addition to the usual training [8], or uses 1850 m for about 30 min with high intensity training [9]. Characteristically, the muscle mitochondrial content, the activity of aerobic enzymes [9, 10, 11] increased. A rise in $\dot{V}o_{2max}$ was also presented [8]. These adaptations can lead to an increase in endurance performance [8]. However, this method may also have no effect on performance at a low altitude [12], or has similar effects as training at a low altitude [9].

Both of these alternative altitude methods may enhance endurance via partially different adaptations. It is therefore of value to find a combination of both, to find whether it may influence haematological, cardiovascular, respiratory and muscular adaptations more than each of them separately. Consequently, endurance performance may be even more enhanced or enhancement may be more reliable than when each of the LHTL or LLTH alternative methods is used. To verify the presented hypothesis, a selection of: a) the moderate altitude of 1500 m for living and training at an altitude of 1000-1500 m (LMTM) method; and b) 1500 m with additional normobaric hypoxia (FiO_2 = 0.18) for living and training at 1000-1500 m (LHTM) were compared in terms of their influences on endurance performance and changes in selected physiological parameters of XC ski-roller runners. Possible differences in adaptations were ascertained.

2 METHODS

Subjects

The subjects in this study participated voluntarily and gave their written informed consent to participate. The study was approved by the National Ethics Medical Committee. A group of 13 XC-ski runners was already trained in the period of the experiment (July), which followed the first preparatory period of basic endurance training (May and June). The group was randomly divided into two parts: subjects who used LMTM (N = 8 subjects, age: 21 ± 3 years, BW: 70 ± 7 kg, BH: 175 ± 8 cm); and subjects (N = 5 subjects, age: 22 ± 5 years, BW: 71 ± 8 kg, BH: 180 ± 5 cm) who used LHTM.

Exercise training and living protocol

The training of both groups was similar. During the altitude training (21 days) the subjects trained for 1-3 sessions/day, including active recovery sessions. The total training volume consisted of 2461 min, without including the strength training volume. All sessions were carried out in a moderate range (1000 – 1500 m) of terrestrial altitude.

Moderate and hypoxic environmental conditions. The subjects using the LMTM method were living at a moderate terrestrial altitude of 1500 m. A hypoxic environmental condition when using the LHTM method was achieved by using 'hypoxic rooms' (b-Cat, Tiel, the Netherlands) in hotel facilities at an altitude of 1500 m (Hotel Planja, Rogla, Slovenia). Normobaric hypoxia was achieved by a vacuum pressure swing adsorption system. The decreased inspiratory oxygen fraction (FiO_2) of 0.18 in addition to the 1500 m terrestrial altitude resulted in a simulated altitude of about 2400-2600 m.

Experimental protocol

The exercise testing consisted of two tests: a trial involving XC-ski roller running and a submaximal cycle ergometer test. Maximal running using cross-country ski rollers on a 6.5 km track of asphalt with an average 7% inclination at a low altitude of about 500-700 m of terrestrial altitude represented four trials: before the altitude training, at the end of the second and third weeks and a week after the subjects had completed the altitude training. The submaximal test [Fig. 1] consisted of a 10 min, 100 W warm up interval, following by a 3 min exercise with additional breathing of a hypoxic gas mixture (FiO_2 = 0.15) and a 3 min interval with normoxic air breathing, all at 100 W. The final test interval consisted of a 3 min, 3.5 W/kg exercise.

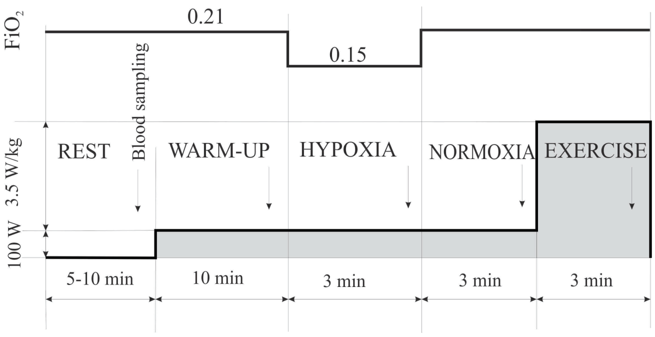

Figure 1: *Schematic presentation of a submaximal test on the cycle ergometer*

Data collection

Vastus lateralis muscle oxygenation: the relative concentration of oxygenated haemoglobin (O_2Hb), deoxygenated haemoglobin (HHb), the tissue oxygenation index (TOI) and total haemoglobin (TOTHb) were continuously monitored by using near-infrared spectroscopy (NIRS), NIRO 200 (Hamamatsu Photonics, Hamamatsu City, Japan). The theory of NIRS is described in [13]. The different path-length factor was assumed to be 3.83. However, due to the uncertainty of this value during exercise the relative values were used. To obtain these values, all measured values were subtracted from the baseline: the average value was obtained during 3 min resting before each test.

3 RESULTS

XC-ski roller trials on the 6.5 km route with a 7% inclination. The average XC-ski rollers' velocities from the four trials showed similar values for both the LMTM and LHTM method and a trend of decreasing over the 3-week experimental period. A week after the end of the experimental period (the 4th trial), the average velocity of the XC-ski roller trial increased relative to the 3rd trial from 3.17 ± 0.30 to 3.44 ± 0.33 m/s (P = 0.01) and was the highest in the experimental period (F = 3.388; P = 0.04) using the LMTM method. The 4th trial velocity using the LHTM method remained similar (3.18 ± 0.30 PRE and 3.35 ± 0.35 m/s POST) to the other velocities (F = 2.739; P = 0.09). Nevertheless, the average velocities did not differ (P = 0.68) between the two groups.

Submaximal cycle ergometer test. During the exercise intervals of warm up and normoxia (both at an intensity of 100 W), HHb and TOTHb decreased [P = 0.02 and P = 0.01, Table 1] when LMTM was compared POST-PRE, but not when LHTM was used. Other observed parameters did not change [Table 1]. No effects on muscle oxygenation were detected in any of the two methods during the hypoxic interval. During the exercise interval, no adaptation of muscle oxygenation parameters TOTHb, HHb, TOI and O_2Hb was observed with the LHTM method [Table 1]. In contrast, the LMTM method enhanced muscle oxygenation [Table 1] during the exercise interval. TOTHb decreased (F = 10.37, P = 0.018) in LMTM after the training period [Table 1]. These changes were accompanied by decreasing HHb values [F = 10.15, P = 0.015, Table 1]. TOI showed a clear tendency of increasing (F = 4.15, P = 0.081). When the training effect was observed only in LMTM, then TOI increased (P = 0.03; Table 4). Training did not influence O_2Hb when the LMTM method was used [Table 1]. In contrast to the LHTM method [Fig. 2], the LMTM method influenced correlated (r = 0.77; P < 0.05) changes between PRE and POST TOTHb (ΔTOTHb) and XC ski-roller velocity (Δv)).

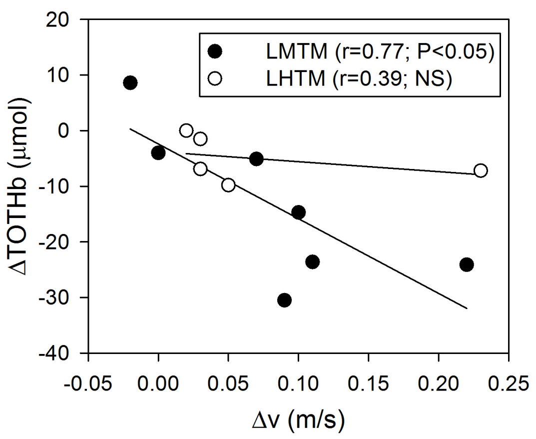

Figure 2: *Correlation between the POST-PRE differences in TOTHb (ΔTOTHb) and XC ski roller velocity (Δv). The increased XC ski roller velocity achieved by LMTM correlated with the larger decrease in TOTHb observed during the submaximal exercise.*

4 DISCUSSION

In this study we ascertained whether two different environmental conditions – living at 1500 m (LMTM) or living at 1500 m with additional normobaric hypoxia (FiO_2 = 0.18%) (LHTM) – differently influenced XC ski-rollers' performance in spite of their similar endurance training. The main findings were: a) XC ski-roller runners who experienced a higher hypoxic dose (LHTM) did not increase their endurance performance in contrast to those who received a lower hypoxic dose (LMTM) and increased their performance; b) during similar absolute submaximal exercise the LHTM method maintained muscle oxygenation via an unchanged HHb and TOTHb in contrast to LMTM, which reduced HHb and TOTHb; c) the decreased muscle blood volume may have even partially increased the endurance performance by using LMTM.

Adaptations to endurance training and living at a moderate altitude (1500 m) observed during the submaximal test on a cycle ergometer. Subjects who used the LMTM method trained at an altitude from 1000 to 1500 m (a moderate altitude). The similar values of O_2Hb and increased TOI showed that those subjects who used this method increased or at least maintained their muscle oxygenation during the submaximal exercise. HHb decreased at a similar O_2Hb. This may be a result of increased exercise economy [5]. The decrease of TOTHb indicates a decrease of muscle blood volume in exercising muscles. It may be achieved by decreasing the blood flow [14], and/or decreasing the blood flow heterogeneity through muscles during the submaximal exercise [15]. The decrease of TOTHb might contribute to the increased XC ski roller trial velocity in our study (Fig. 2) when the LMTM was used.

Adaptations to endurance training and living in hypoxic environmental conditions (1500 m with additional normobaric hypoxia of FiO_2 = 0.18) (LHTM) observed during the submaximal test on a cycle ergometer. LHTM did not influence muscle oxygenation: TOTHb only showed a tendency of decreasing, while HHb and TOI revealed a tendency of increasing it. Despite this, it is possible that muscle oxygenation was not influenced by LHTM; it is more likely that the resulting oxygenation was a balance between two opposite effects: increased perfusion by hypoxia (number of opened capillaries) [16] and decreased blood flow and blood flow heterogeneity [14, 15]. This phenomenon is presented in the absence of a correlation between the changes of TOTHb which are minimal and did not correlate with the changes in the XC ski roller velocities (Fig. 2). This is a different outcome to when the LMTM method is observed.

5. CONCLUSION

The moderate altitude of 1500 m selected in our study and the relative large training volume at a moderate altitude of 1000-1500 m (LMTM method) mainly increased muscle oxygenation by decreasing deoxygenated haemoglobin (HHb) and the muscle blood volume (TOTHb), which seems to at least partially influence an enhanced endurance performance. When this method increased its hypoxic dose by an additional FiO_2 = 0.18 to the sleeping altitude of 1500 m (LHTM method), this influenced neither the endurance and muscle oxygenation nor the haematological characteristics and $\dot{V}O_{2max}$.

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Table 1
BLOOD AND MUSCLE OXYGENATION RESPONSES DURING TESTING ON THE CYCLE-ERGOMETER

			Rest	Warm-up (100 W, 10 min)	Hypoxia (100 W, 3 min, $FiO_2 = 0.15$)	Normoxia (100 W, 3 min)	Exercise (3.5 W/kg, 3 min)
LMTM	O_2Hb (µmol)	PRE	0	2.8 ± 4.1	2.1 ± 4.9	4.4 ± 4.7	-10.8 ± 7.3
		POST	0	-0.7 ± 7.1	-0.5 ± 6.7	1.7 ± 7.2	-6.9 ± 7.8
	HHb (µmol)	PRE	0	4.5 ± 4.6	8.5 ± 4.6	6.4 ± 4.6	24.8 ± 10.1
		POST	0	-0.4 ± 5.2 *	1.4 ± 5.3 **	0.2 ±5.2 **	10.6 ± 12.2 *
	TOI (%)	PRE	0	-1.6 ±3.7	-3.6 ± 3.7	-2.4 ± 3.6	-11.5 ± 6.5
		POST	0	-1.2 ± 2.3	-1.5 ± 2.1	-0.6 ± 2.3	1.0 ± 8.5 *
	TOTHb (µmol)	PRE	0	8.0 ± 5.3	10.4 ± 5.1	11.6 ± 5.7	13.8 ±7.0
		POST	0	-2.3 ± 12.3 *	-0.1 ± 11.8 **	0.9 ± 4.4 **	1.7 ± 12.9 *
LHTM	O_2Hb (µmol)	PRE	0	0.8 ± 4.5	3.5 ± 4.2	5.9 ± 3.4	-2.4 ± 7.0
		POST	0	1.2 ± 2.3	3.1 ± 3.0	4.9 ± 4.2	-0.7 ± 4.6
	HHb (µmol)	PRE	0	1.5 ± 2.6	3.8 ± 5.9	3.2 ± 4.3	16.7 ± 10.4
		POST	0	-1.9 ± 2.6	-0.9 ± 3.0	-1.2 ± 3.1	8.0 ± 4.0
	TOI (%)	PRE	0	-2.6 ±1.5	-3.0 ± 3.3	-1.4 ± 3.2	-11.1 ± 6.4
		POST	0	1.9 ± 2.7	1.9 ± 2.9	2.1 ± 3.7	-1.4 ± 7.2
	TOTHb (µmol)	PRE	0	1.4 ± 5.6	8.1 ± 6.8	9.2 ± 4.3	18.5 ± 5.2
		POST	0	0.7 ± 3.7	3.8 ± 3.0	5.8 ± 3.0	7.4 ± 3.7

* - $P < 0.05$ (PRE-POST difference) ** - P = < 0.01 (PRE-POST difference)

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COOLING EFFECT OF DIFFERENT »COOLING« SPORT SHIRTS BY CYCLE TEST ON ERGOMETER: A CASE STUDY

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Background The aim of the research was to determine the effect of the cooling ("ice") shirts on the temperatures measured on the lower back region of an athlete during intense bicycle training. The incorporated technical fibres had a remarkable feeling when the participants put their shirts on the body.

Methods Three different technical fabrics (shirts) were investigated. The fabric selection was performed by Julon (Fabric Textile Company). We called it "ice shirt 1 (with maximal cooling impregnation), "ice shirt2" (with half cooling impregnation); "sport shirt" (the same shirt without impregnation). Additional we added also the protocol, where the participant did the test without shirt. For the purpose of the research we did 4 different protocols of the step test on the Lode cycle ergometer. Test protocol was named "the modification of Conconi test". The test started with 20W, where the intensity increased every minute for 20W. For the test we had a very good recreational triathlete (male 25 years; 175cm: 68kg) and we expected that he will be able to achieve the expected level of the stress test on a Lode cycle ergometer.

Results The following parameters were monitored during the test: (1) the heart rate and (2) the temperature on the surface of the skin was measured (average and maximum temperature at the lower back. The results of the test shown us that there were some differences between the average temperatures at the surface of the shirts, when we used different cooling shirts.

Conclusion However for better results we need to improve our protocols in sense of similar terms and conditions. We need to invite more participants and find the way how to randomly using a variety of (cooling) T- shirts. Of course we want to continue with more analysis and researches about the cooling effect of specific sport shirts. What we don't know and it might be good to takes into account is the fabric density by different shirts. The most important suggestion might be that in the future it will be better to focus more on core temperature of the body. We could expect when we will measure the core temperature of the body with the temperature on the surface of the shirts it will show us more accurate and credible results.

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