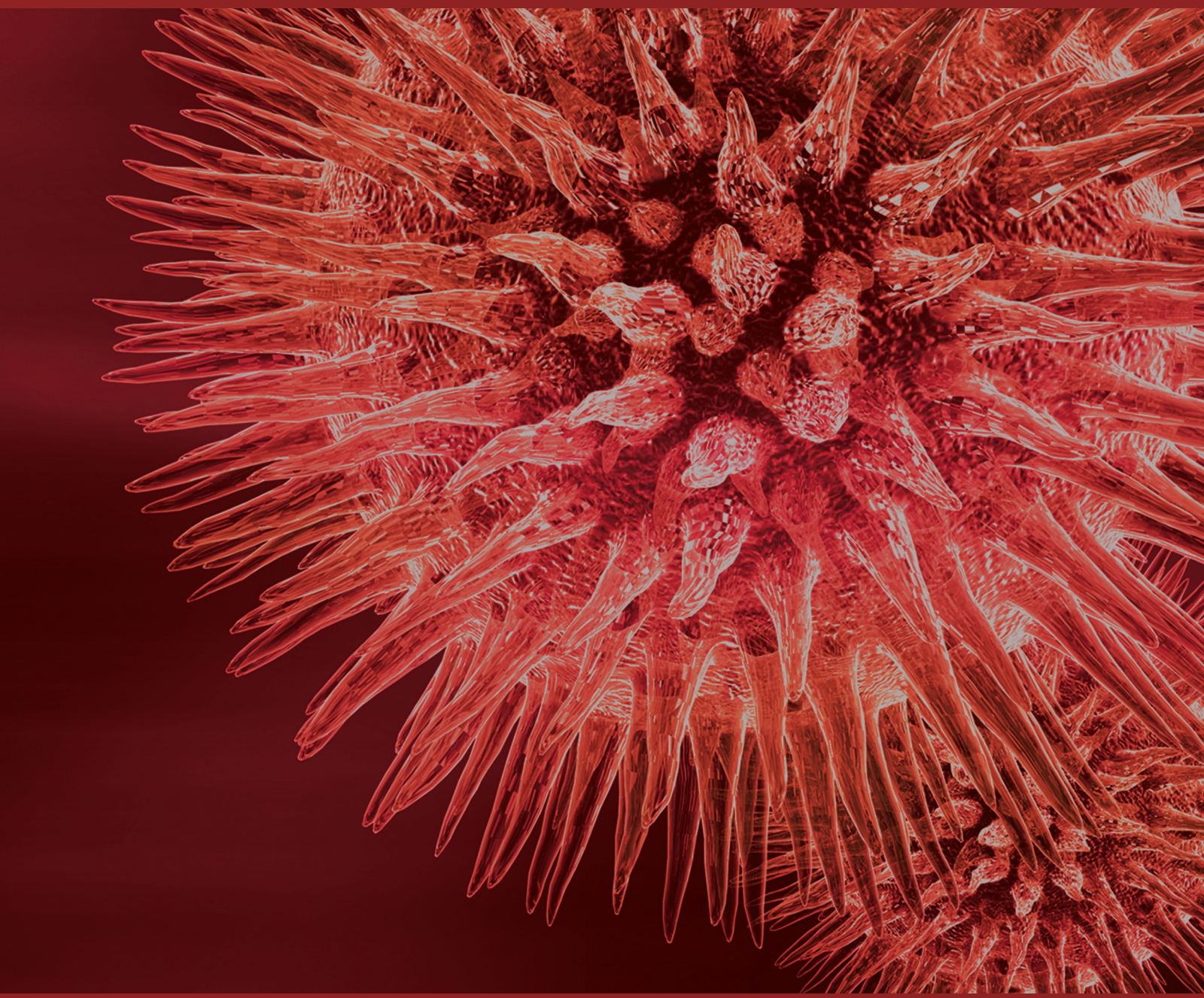
BioMed Research International

Exercise Physiology, Cognitive Function, and Physiologic Alterations in Extreme Conditions 2016

Guest Editors: Ellen L. Glickman, Edward J. Ryan, and David Bellar



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Contents

Hindawi Publishing Corporation
BioMed Research International
Volume 2016, Article ID 7640898, 1 page
http://dx.doi.org/10.1155/2016/7640898

Editorial

Exercise Physiology, Cognitive Function, and Physiologic Alterations in Extreme Conditions 2016

Ellen L. Glickman,[1] Edward J. Ryan,[2] and David Bellar[3]

[1]*School of Health Science, Exercise Physiology, Kent State University, Kent, OH 44242, USA*
[2]*Exercise Science, Chatham University, Pittsburgh, PA 15232, USA*
[3]*School of Kinesiology, University of Louisiana at Lafayette, Lafayette, LA 70506, USA*

Correspondence should be addressed to Ellen L. Glickman; eglickma@kent.edu

Received 30 October 2016; Accepted 31 October 2016

The field of *exercise physiology* is a broad field of inquiry that focuses on the total physiological responses of humans to stressors such as exercise or the environment. While it is well known that extremes environments impose unique stressors on humans that manifest profound physiological effects, it remains an area that is underreported upon in the literature. It should be noted that it is often through the study of physiological extremes that the scientific community gains greater insight into the capacity of the human organism for physiological function.

Furthermore, it has been shown that extreme environments have a detrimental effect on cognitive function and also result in elevated inflammation and changes in metabolism. Though not commonly experienced by all people, there are instances either occupationally or recreationally where people not only are exposed to extreme environments, but also are required to compound the stress through exercise. The resulting combination can manifest even greater physiological responses, which require investigation.

The editors invited contributions for this special edition that represented current and timely expansion of knowledge in the areas of environmental and exercise physiology in extreme conditions. It is of utmost importance that inquiry in this area continues so that a more comprehensive understanding of the human physiological response to unique environments can be developed and methods for coping with these environmental stressors improved.

Ellen L. Glickman
Edward J. Ryan
David Bellar

Hindawi Publishing Corporation
BioMed Research International
Volume 2016, Article ID 6245609, 9 pages
http://dx.doi.org/10.1155/2016/6245609

Research Article

Acute Mountain Sickness Symptoms Depend on Normobaric versus Hypobaric Hypoxia

Dana M. DiPasquale,[1] Gary E. Strangman,[1] N. Stuart Harris,[2] and Stephen R. Muza[3]

[1]*Department of Psychiatry, Massachusetts General Hospital, Harvard Medical School, Charlestown, MA, USA*
[2]*Division of Wilderness Medicine, Department of Emergency Medicine, Massachusetts General Hospital, Harvard Medical School, Boston, MA, USA*
[3]*Thermal and Mountain Medicine Division, U.S. Army Research Institute of Environmental Medicine, Natick, MA, USA*

Correspondence should be addressed to Dana M. DiPasquale; dipasquale.dana@gmail.com

Received 20 May 2016; Revised 13 September 2016; Accepted 21 September 2016

Academic Editor: Edward J. Ryan

Acute mountain sickness (AMS), characterized by headache, nausea, fatigue, and dizziness when unacclimatized individuals rapidly ascend to high altitude, is exacerbated by exercise and can be disabling. Although AMS is observed in both normobaric (NH) and hypobaric hypoxia (HH), recent evidence suggests that NH and HH produce different physiological responses. We evaluated whether AMS symptoms were different in NH and HH during the initial stages of exposure and if the assessment tool mattered. Seventy-two 8 h exposures to normobaric normoxia (NN), NH, or HH were experienced by 36 subjects. The Environmental Symptoms Questionnaire (ESQ) and Lake Louise Self-report (LLS) were administered, resulting in a total of 360 assessments, with each subject answering the questionnaire 5 times during each of their 2 exposure days. Classification tree analysis indicated that symptoms contributing most to AMS were different in NH (namely, feeling sick and shortness of breath) compared to HH (characterized most by feeling faint, appetite loss, light headedness, and dim vision). However, the differences were not detected using the LLS. These results suggest that during the initial hours of exposure (1) AMS in HH may be a qualitatively different experience than in NH and (2) NH and HH may not be interchangeable environments.

1. Introduction

Unacclimatized individuals rapidly traveling to high altitude are at risk for developing acute mountain sickness (AMS), an illness of nonspecific symptoms including headache, nausea, vomiting, fatigue, anorexia, and dizziness. Symptoms typically start 2–12 hours following altitude exposure [1, 2]. While AMS is not life-threatening, symptoms can be disabling, causing considerable discomfort and disrupting activity. Presence and severity of AMS are most commonly assessed with two subjective Likert-style questionnaires. The Environmental Symptoms Questionnaire (ESQ) is a 67-weighted-item inventory of expected physiological and psychological symptoms developed by the US military [3, 4]. A subset of this inventory with questions related to cerebral function (AMS-C) has been validated against the full ESQ inventory [5] and is commonly used to assess AMS [6]. The second questionnaire, developed by a consensus committee, consists of five self-reported items and is known as the Lake Louise Self-report (LLS) [7]. There is no single gold-standard assessment tool [8, 9] and, unfortunately, the two questionnaires do not always produce the same diagnosis [10].

Early work defining AMS demonstrated that the most prevalent symptoms were headache and insomnia followed by various others, depending on those investigated in the study [11, 12]. Since the development of the ESQ and LLS, though, there has been limited research examining the prevalence of symptoms within each questionnaire or between questionnaires, particularly at the beginning of hypoxia exposure. Although questionnaires assessing the presence of AMS were developed for use in hypobaric hypoxia (HH)—that is, high altitude in the mountains or in a hypobaric chamber—they have been adopted to also measure AMS under conditions of simulated high altitude using normobaric hypoxia

Table 1: Subject characteristics. None of the subject characteristics were different among groups ($p > 0.05$). Data are expressed as means ± SD.

Environment	NN		NH		HH	
Exercise	10 min	60 min	10 min	60 min	10 min	60 min
Sex (n)	M = 6, F = 6	M = 6, F = 6	M = 6, F = 6	M = 6, F = 6	M = 5, F = 6	M = 7, F = 6
Age (y)	24.4 ± 4.2	30.6 ± 8.4	28.5 ± 10.0	25.1 ± 4.9	30.5 ± 8.3	26.9 ± 7.1
Height (cm)	172.0 ± 6.9	172.0 ± 6.4	171.5 ± 8.6	167.9 ± 10.4	170.9 ± 9.7	174.0 ± 6.6
Weight (kg)	68.7 ± 8.7	66.8 ± 8.2	68.3 ± 12.1	67.5 ± 12.0	71.3 ± 9.8	70.7 ± 10.3
HR_{rest} (bpm)	65.6 ± 7.1	62.2 ± 11.0	62.3 ± 12.6	61.3 ± 10.6	60.0 ± 9.1	62.0 ± 9.2

(NH). Positive scoring of AMS based on these questionnaires led to the conclusion that AMS is present in NH as well as HH [13]. This skipped a crucial step, however: determining if the known AMS symptoms due to HH are the same as those in NH.

Traditionally, AMS has been thought to primarily be the result of hypoxia. Emerging data, however, suggests that not only hypoxia, but also the hypobaria of high altitude contributes to the development of AMS [14–18]. Recently, AMS prevalence and severity have been observed to be higher in HH than NH [2, 18]. Evidence is also mounting that the two conditions may produce different performance and physiological effects as well [14, 15, 17, 19, 20]. Despite evidence supporting this, to our knowledge, no studies have examined potential differences in symptoms in the two environments.

We hypothesized that if NH and HH have different prevalence and severities of AMS, the symptoms experienced in NH and HH may also be different. Therefore, we compared the AMS symptoms most influential in AMS diagnosis in NH versus those in HH. We also compared the symptoms reported with LLS and those reported with ESQ as the two questionnaires have different diagnostic criteria and survey both similar and different symptoms.

2. Materials and Methods

2.1. Subjects. Thirty-six healthy subjects (Table 1) volunteered and were selected after screening to participate in this study approved by the Institutional Review Boards of the Massachusetts General Hospital and US Army Research Institute of Environmental Medicine. Subjects were regular exercisers born at <2134 m, living in areas that were <1220 m, and had not traveled to areas that were >1220 m for more than 2 d in the last 2 mo. After providing written informed consent, subjects were medically cleared following a clinical exam.

2.2. Overall Design. As part of a larger study on physiological differences between NH and HH, each subject was randomly assigned to 2 of 6 possible groups. Groups were defined by 3 environments crossed with 2 exercise durations: that is, normobaric normoxia (NN), NH, and HH crossed with short exercise (10 min) and long exercise (60 min). This was a partial repeated-measures design; having subjects participate in all 6 conditions maximizes power but was deemed impractical from both retention and potential condition-carry-over-effects perspectives. Having each subject participate in only 1 condition (fully between-subjects design) greatly reduces power due to between-subject variability. Intermediate cases (participating in 2–5 conditions) represent compromises between power and subject retention. Statistical power was further optimized—and bias minimized—by having fully counterbalanced condition-pairs and orders, resulting in 12 exposures per condition. None of the subject characteristics were different among groups ($p > 0.05$). The different exercise durations allowed for varying AMS severities among our sample [18] without reducing power since our analysis, described below, did not separate data based on exercise duration. None of the subject characteristics were different among groups ($p > 0.05$), which were fully counterbalanced condition-pairs, orders, and sexes (Table 1).

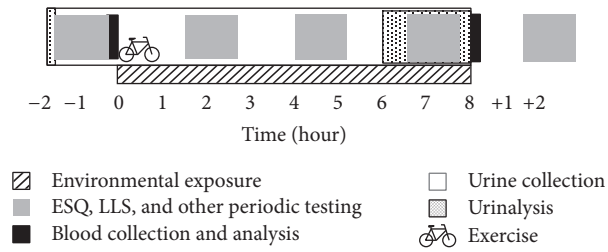

Figure 1: Schematic of experimental timeline. Subjects were exposed to 8 h of normobaric normoxia, normobaric hypoxia, or hypobaric hypoxia. Various physiological measurements were made before, throughout, and after testing. The ESQ and LLS were administered before exposure, 3 times over the exposure period, and after exposure.

Subjects performed sea-level testing, ascended (~15 min), exercised, spent 8 h in the environmental condition with periodic testing, and were tested again at sea level (Figure 1). Periodic testing included ~75 min battery of measurements (i.e., noninvasive cerebral and systemic physiology and cognition) including the ESQ and LLS, which were administered at the beginning of each period. Testing was performed 1.5 h before exposure, at 1.5, 4, and 6.5 h into exposure, and 1.5 h after exposure. In between testing bouts, subjects were permitted to rest, read, listen to music, or watch movies for ~75 min. Subjects were advised not to consume alcohol or exercise 24 h prior to testing. Regular coffee drinkers were permitted their usual morning beverage prior to testing. Subjects were provided food and caffeine-free drinks *ad libitum* for the remainder of the day. Two weeks separated testing days.

2.3. Environmental Exposures. Subjects were naive to the assigned conditions. They were not provided with any information about which room was for NN, NH, or HH, and all research personnel used supplemental oxygen regardless of the condition. NN was performed in the hypobaric chamber at barometric pressure (P_B) = 752 mmHg, which enabled secure sealing of the chamber door, further ensuring subject naivety (partial pressure of inspired oxygen (P_{IO_2}) = 147.3 mmHg; 300 m equivalent altitude). HH was performed in a hypobaric chamber (P_B = 439 mmHg; P_{IO_2} = 81.9 mmHg; 4400 m equivalent altitude). NH was performed at ambient pressure in a hard vinyl-sided hypoxia room (Colorado Altitude Training, Boulder, CO) with ambient oxygen partial pressure matched to the HH condition at 91.7 mmHg (P_B = 760 mmHg; P_{IO_2} = 86.1 mmHg; 4400 m equivalent altitude). Following all testing, >90% of subjects could not guess or incorrectly guessed their experimental condition.

2.4. Exercise. As described elsewhere [18], following ascent, subjects performed moderate exercise as a stimulus to accelerate the AMS process and vary the severity of AMS. Briefly, cycling was performed at 52.1 ± 4.4% of heart rate reserve (Excalibur Lode, Groningen, The Netherlands) and was completed within the first hour of exposure.

2.5. Questionnaires. Symptom presence and severity were measured using a 19-question subset of the ESQ, including all of the cerebral symptoms (AMS-C) as well as those to assess fatigue and alertness, and 4 of the 5 LLS symptoms (Table 2). The LLS question assessing sleep quality was not included since subjects did not experience a night at simulated altitude. Using the ESQ, having AMS (AMS_E+) was defined as an AMS-C score ≥ 0.7 with current or recent "altitude" exposure [5]. Using the LLS, having AMS (AMS_L+) was defined as the presence of a headache, at least one other symptom, a cumulative score of 3 or greater, and recent exposure to "altitude" [7]. By definition, a subject in NN—either during pre-exposure testing periods or in the NN blinded condition—cannot have AMS but can present with symptoms assessed by the questionnaire as a result of boredom, fatigue, and frustration and other symptoms related to any very long day of testing; as such, all AMS diagnoses in NN were considered AMS−, but individual symptoms' severities were still rated by subjects to account for those non-AMS-related symptoms. Out of 360 observations, there were only 2 instances when a subject in NN had a symptom score meeting the criteria for AMS+. Thus, the inclusion of unrelated symptoms allows us to accurately describe the symptoms that directly related to having AMS. AMS+/− classification was designated at each time point the questionnaires were administered, such that an individual could be AMS− early in the exposure and may not be AMS+ until later.

2.6. Data Analysis. Since all pre-exposure measurements were conducted in NN, they were all included in the NN analysis. Post-exposure measurements made after NH and HH were included with the NH and HH analysis, respectively.

We used classification trees to identify symptoms most influential for identifying AMS+ in NH versus HH. Decision trees are a simple but extremely useful form of multiple variable analysis used in numerous fields. In medicine, categorical and regression tree analysis (CART) employs a hierarchical selection of the most influential diagnostic criteria. For example, since the seminal work on CART [21] described a tree identifying high risk patients for myocardial infarction, hundreds of studies have used CART analysis in clinical settings investigating risk and diagnosis of heart attacks.

CART is a binary recursive partitioning method whereby data are successively split along two axes—"branches"—of the explanatory variables (i.e., presence or absence of a symptom) so that at each node the symptom is chosen that maximally distinguishes the response variable (AMS+/−) in the right and left branches [21]. Branches splitting to the right indicate the presence of the symptom, and branches to the left indicate the absence of the symptom. To implement our analysis, a tree was generated with the CART "rpart" package version 4.1-9 [22] for R version 3.1.2, which determined the symptom with the optimal first split, that being the one with the greatest gain in purity at a node (creating groups of observations that are as closely related as possible, i.e., as many AMS+ observations and fewest AMS− observations as possible or vice versa), assessed by the improvement in the Gini diversity index [21].

Table 2: Symptoms measured by LLS and ESQ included in the analyses.

Question	Symptom	AMS-C score weighting factor
ESQ 1	I feel lightheaded	0.489
ESQ 2	I have a headache	0.465
ESQ 3	I feel dizzy	0.446
ESQ 4	I feel faint	0.346
ESQ 5	My vision is dim	0.501
ESQ 6	My coordination is off	0.519
ESQ 7	I feel weak	0.387
ESQ 8	I feel sick to my stomach (nauseous)	0.347
ESQ 9	I lost my appetite	0.413
ESQ 10	I feel sick	0.692
ESQ 11	I feel hungover	0.584
ESQ 12	I feel tired	—
ESQ 13	I feel sleepy	—
ESQ 14	I feel thirsty	—
ESQ 15	I have a runny nose	—
ESQ 16	My vision is blurry	—
ESQ 17	My concentration is off	—
ESQ 18	My eyes feel irritated	—
ESQ 19	I am short of breath	—
LLS 1	Headache	—
LLS 2	Gastrointestinal symptoms (nausea, loss of appetite, vomiting)	—
LLS 3	Fatigue and/or weakness	—
LLS 4	Dizziness/lightheadedness	—

Table 3: Summary of symptoms leading to AMS+ listed in order of importance. Approximate importance scores are in parentheses.

All		NH		HH	
AMS_E+	AMS_L+	AMS_E+	AMS_L+	AMS_E+	AMS_L+
Dizzy (49)	Headache (48)	Sick (10)	Headache (37)	Faint (37)	Headache (56)
Sick (14)	Dizzy/lightheaded (23)	Shortness of breath (9)	Dizzy/lightheaded (17)	Appetite loss (7)	Dizzy/lightheaded (8)
Headache (5)	—	—	—	Lightheaded (3)	—
Coordination off (3)	—	—	—	Dim vision (2)	—

The generation of splits stopped when no significant decrease of the impurity was achieved or the sample size was less than $n = 20$. The analysis conducted as many splits as possible, and then 10-fold cross-validation was performed to identify the optimal model with good generalizability [23]. This was accomplished by splitting the data into 10 roughly equal parts, each containing a similar distribution for AMS+. First, 9/10 of the data was used to grow the tree, and the remaining 1/10 was used as a test sample to obtain initial estimates of the tree's error rates. This was repeated 10 times such that a different 1/10 of the data was used as the test sample. The 10 minitests were combined to form error rates for trees of each possible size. The error rates were then applied to the trees based on the entire learning sample, and the trees were pruned to the lowest possible error rate [24].

To determine if different symptoms existed depending on the environment, we created 3 classification trees: (1) all environments combined, (2) NN + NH, and (3) NN + HH. Observations in NN were included in every tree as a control for non-hypoxia-related symptoms. Three trees were created for each AMS questionnaire used (LLS or ESQ), resulting in a total of six trees. Those trees with an AMS diagnosis based on the ESQ criteria were split on the presence or absence (*not severity*) of symptoms without weighting any AMS-C symptoms, and those with a diagnosis based on LLS were split on the presence of absence of LLS symptoms.

3. Results

The diagrams of the tree structures created using the ESQ criteria for AMS diagnosis are presented in Figure 2. In all environmental conditions combined (Figure 2(a)), the classification tree had five terminal nodes. Two of the five nodes were classified as AMS_E+ and three were AMS_E− based on the distribution of AMS_E+ and AMS_E− observations; if the majority of observations in a node are AMS+, then the node is classified as AMS+. There were 57 observations of AMS_E+ and 48 of them were captured in terminal nodes 4 and 8. There were 302 AMS_E− observations, and terminal nodes 1, 5, and 7 captured 296 of them. This classification identified the following symptoms as the greatest risk factors for AMS_E+, in order of importance: dizziness, feeling sick, headache, and coordination being off (Table 3).

In NH, AMS_E+ was most heavily influenced by feeling sick and shortness of breath (Figure 2(b)), in order of importance (Table 3). The classification tree had 3 terminal nodes, 1 of which was classified as AMS_E+ and the other 2 as AMS_E−. There were 15 observations of AMS_E+, and 10 were captured in terminal node 4. There were 219 AMS_E− observations, and all 219 were captured in terminal nodes 1 and 3.

In HH, the classification tree had 6 terminal nodes, 3 of which were AMS_E+ and 3 were AMS_E− (Figure 2(c)). There were 42 observations of AMS_E+, and 35 were captured in terminal nodes 6, 8, and 10. There were 203 AMS_E− observations, and 196 were captured in terminal nodes 3, 7, and 9. AMS_E+ observations in HH were most influenced by feeling faint, appetite loss, lightheadedness, and having dim vision, in order of importance (Table 3).

The diagrams of the tree structures created using the LLS criteria for AMS diagnosis are presented in Figure 3. All three classification trees had three terminal nodes. One of the three nodes was classified as AMS_L+ and the other two were AMS_L−. In all conditions combined, 48 of the 56 AMS_L+ observations were captured in node 4. The other terminal nodes captured 297 of the 303 observed AMS_L− observations. These classification trees all identified the following symptoms as the greatest risk factors for AMS_L+, in order of importance: headache and dizzy/lightheaded (Table 3).

4. Discussion

We compared AMS symptoms in NH with HH, and we compared symptoms assessed by the ESQ versus LLS during the early stages of hypoxia exposure. Using a novel method for examining AMS, we progressively built up a model containing the set of symptoms that most contributed to AMS+. We found differences in NH and HH produced symptoms during an 8 h exposure, though this depended on the use of LLS or ESQ. When querying a broad set of symptoms from the ESQ, NH had fewer and different symptoms contributing to AMS+ than HH, but the LLS was not able to detect these differences. Using ESQ, AMS_E+ in NH was characterized by feeling sick and shortness of breath, while, in HH, AMS_E+ was primarily influenced by feeling faint, having loss of appetite, feeling lightheaded, and having dim vision. Using LLS, however, AMS_L+ individuals experienced headache and feeling dizzy/lightheaded whether the data was separated by hypoxia condition or not.

With many potential symptoms, subjective questionnaires, different Likert scales for assessing severity, and various weights applied to symptoms, it is difficult to determine

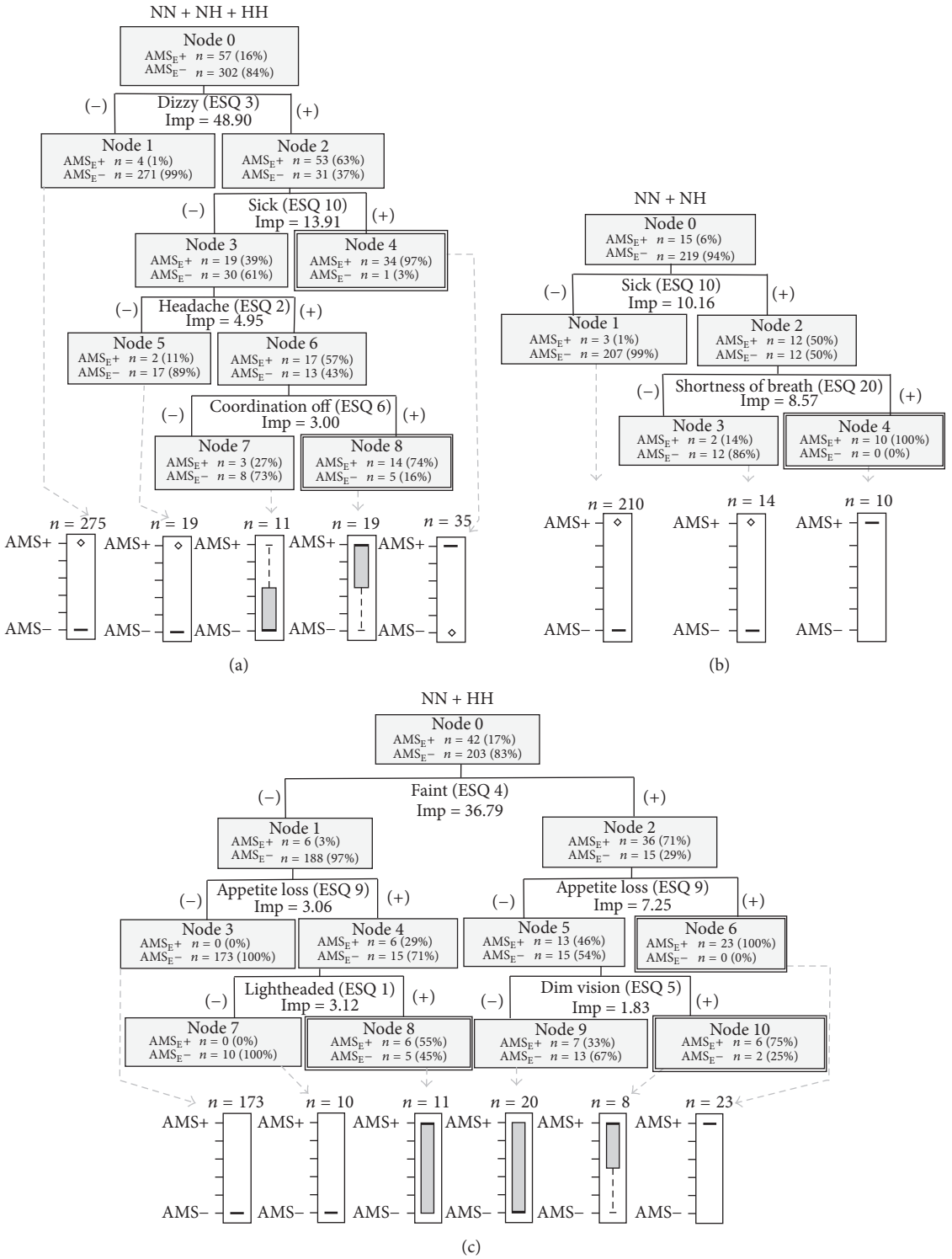

Figure 2: Classification trees based on ESQ symptoms and AMS$_E$+ determination by AMS-C score in (a) all conditions combined, (b) NH, and (c) HH. CART analysis produces decision trees. The trees are created by splitting branch after branch. Branches were split based on the symptom listed at each node in the tree. The symptom was chosen by the CART analysis based on the importance of the symptom in determining AMS+. This importance is calculated using the Gini diversity index. If a subject had the symptom, he/she was placed in the right-hand branch. If the symptom was absent, he/she was placed in the left hand of the branch. If a majority of cases in a single node were AMS+, then the node was classified as AMS+. The double outlined terminal nodes indicate those classified as AMS$_E$+ based on the box plot distributions of prediction results which are under each tree. The box plots are another graphical representation of the distribution of subjects classified closer to either AMS+ or AMS−. The findings from these trees are summarized in Table 3. Imp = importance score.

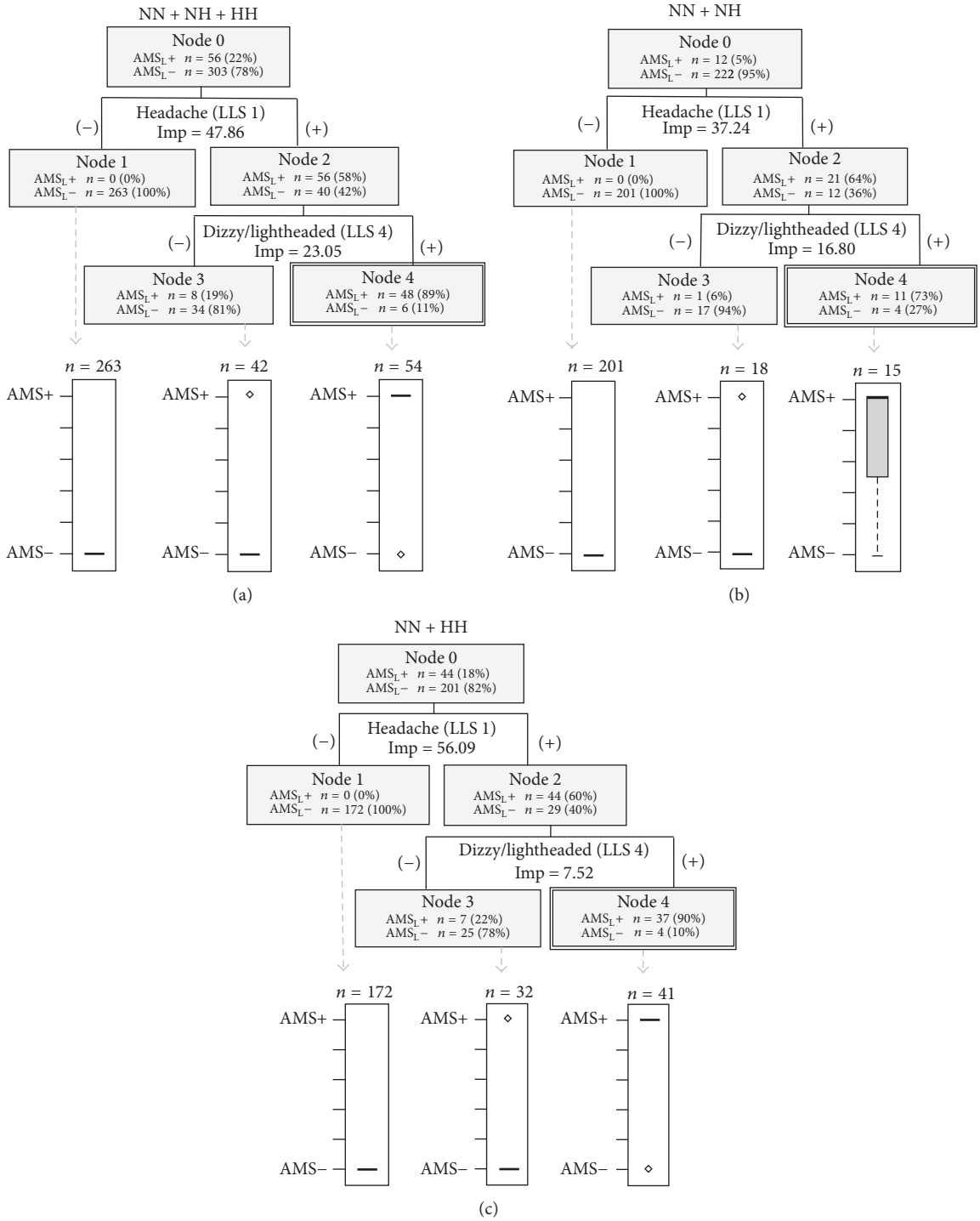

Figure 3: Classification trees based on LLS symptoms and AMS$_L$+ determination by LLS criteria in (a) all conditions combined, (b) NH, and (c) HH. Double outlined terminal nodes indicate those classified as AMS$_L$+ based on the box plot distributions of prediction results. Imp = importance score.

if AMS in NH is on the same continuum as AMS in HH and just less severe, or if AMS in NH is characterized by different symptoms than in HH. We performed tree analysis, a useful tool used in many fields of study, to make yes/no decisions about which symptoms are most important for classifying a condition, specifically the early stages of AMS. In many clinical models, a decision tree can have its branches split on the severity of a clinical sign, such as blood pressure >100 mmHg or body mass index >30, for example. However, using the severity of individual AMS symptoms to split branches would

likely create a confusing tree that is difficult to interpret and, therefore, fail to answer the basic question—which symptoms most contribute to AMS+ in NH versus HH? Consequently, we used tree analysis by splitting branches based on the presence or absence of symptoms regardless of the severity or weight of the symptoms.

Our first tree analysis determined the symptoms most important to AMS_E+, based on the Gini diversity index of importance, with the traditional assumption that AMS symptomatology is the same regardless of the environment. When combining all environments together, AMS_E+ was mostly influenced by dizziness, feeling sick, having a headache, and coordination being off, in order of decreasing importance. A majority (~60%) of all AMS_E+ observations could be correctly identified as having AMS if subjects experienced dizziness and feeling sick, and if a subject was experiencing both of these symptoms, he/she had a 97% chance of having AMS_E+. Interestingly, not all of the identified symptoms are the most heavily weighted symptoms for determining the AMS-C score and ultimately the AMS_E+ diagnosis. This suggests that our trees are not confounded by the differential weighting of symptoms which may have been observed if modeling trees with the severity of a symptom instead of presence/absence of a symptom.

In NH only, feeling sick and shortness of breath characterized AMS_E+ subjects. In NH, feeling sick had the largest importance whereas, in all conditions combined, feeling sick ranked second to feeling dizzy. Subjects who did not feel sick in NH had a 99% chance of being AMS_E−. Subjects who both felt sick and had shortness of breath had a 100% chance of having AMS_E+. Although feeling sick is the most heavily weighted cerebral symptom, this was the only tree in which a non-AMS-C symptom—shortness of breath—was also important.

Compared to the fairly simple tree in NH using the ESQ, HH produced more branches and symptoms, including feeling faint, loss of appetite, lightheadedness, and dim vision, in order of importance. None of the symptoms in individuals with AMS_E+ in NH were the same as those in HH, suggesting that the experience of subjects may have been qualitatively different during the initial 8 h of exposure to hypoxia. Whether or not the overall complexity of the HH tree is because of the branching decisions chosen for CART, because HH produced a more complicated illness, or because a greater AMS severity in HH was the result of numerous additive symptoms cannot be determined from this analysis. The overall sensitivity of the tree (~83%) was similar to the tree combining all conditions, suggesting the HH condition is masking the NH condition in the all-conditions-combined tree. The specificity of the HH tree was high (~97%) with many pure nodes, again showing that this method could be useful in identifying AMS_E− subjects. Importantly, subjects who experienced both feeling faint and appetite loss had a 100% chance of having AMS_E+. Likewise, subjects who felt faint and had dim vision had a 75% chance of having AMS_E+. Therefore, though the overall accuracy of the tree was fair, it might be used as a quick prescreen to isolate individuals with a high likelihood of having AMS_E+ based on just two reported symptoms. More interestingly, perhaps, HH symptoms most important in the early stages of AMS_E+ included only cerebral symptoms, compared to NH which also included a noncerebral symptom. It is possible that a longer exposure would allow symptoms to evolve in NH and HH, such that eventually AMS symptoms in the two environments become similar. However, this remains to be determined in a controlled trial comparing NH and HH and individual symptoms.

Traditionally, AMS is thought of as a nonspecific group of symptoms with headache as a primary symptom. Our results indicated that although headache was a predictor in all-conditions-combined, environment-specific trees (NH or HH), using ESQ did not include headache as a major contributor to AMS_E+ during the initial stages of AMS. This suggests that when combining conditions, headache may appear more important than it really is. This lends support to the camp of altitude researchers who prefer the ESQ assessment tool because it does not preclude the presence of AMS if there is no headache [25]. In contrast to ESQ, LLS requires the presence of a headache for subjects to be considered AMS_L+ [7]. This is evident in the LLS classification trees as all had an initial split on headache, sending all headacheless subjects to a terminal AMS_L− node. All trees, regardless of environment, indicated that headache and dizziness/lightheadedness characterized AMS_L+. Because this characterization is different than that using a broader set of symptoms, as in the ESQ, we speculate that the LLS is not able to identify variations in AMS symptoms in the early stages of AMS. Neither dizziness nor lightheadedness was a symptom identified in NH using the ESQ, despite these symptoms being queried by both the ESQ and LLS, again suggesting LLS may not be as useful in NH as ESQ during short-term hypoxia exposure. Additionally, the use of compound symptoms in the LLS may obscure any differences as individuals who feel one symptom but not the other are unable to differentiate this on the questionnaire. Collectively, this implies that future research on early AMS symptoms in NH should be particularly cognizant of the questionnaire used for assessment.

There are three main limitations to this study. This first is that the NH and HH conditions were matched on P_{O_2}, causing a 4.2 Torr difference in P_{IO_2} between hypoxic conditions. Although not possible here due to the nature of CART, in our other analyses of the physiological data collected in this study [18, 20], we used oxygen saturation (Sp_{O_2}) as a covariate to account for this extremely small difference in P_{IO_2}, since Sp_{O_2} represents the overall functional output of ventilation and pulmonary gas exchange. In all instances, we found that adding Sp_{O_2} as a covariate to our models did not markedly change the magnitude of regression coefficients or their significance, ultimately suggesting that NH and HH produced different responses. Therefore, the difference in P_{IO_2} between NH and HH is likely negligible. The second limitation is the cyclic nature of assessing the AMS symptoms that contribute to the AMS diagnosis when using those same symptoms to diagnose AMS. Unfortunately, though, there is currently no objective way to determine AMS+. As such, this cyclic nature is inherent. This can be observed in the findings of the LLS trees in which headache, a symptom

required for AMS_L+, was present in all trees. However, we queried numerous symptoms from the ESQ but only used the AMS-C symptoms for the AMS_E+ classification, reducing the cyclic nature. Additionally, we divided branches of the trees based on the presence or absence of *unweighted* symptoms—not *weighted* severity scores—also reducing the cyclic nature. In fact, this was evident in our findings, in which the NH tree included non-AMS-C symptoms. Future work can further reduce the cyclic nature by using all 67 of the ESQ symptoms instead of a subset and continuing to use only the AMS-C symptoms to classify AMS_E+. Finally, because AMS symptoms may evolve over longer durations especially after a poor night's sleep, the results of the present analysis are limited to the first third of a day of hypoxia exposure.

5. Conclusions

In summary, using CART to determine the symptoms that contributed most to early AMS+, we found that NH and HH did not produce common symptoms when evaluated with a range of symptoms queried by the ESQ. Conversely, using a small set of mostly compound symptoms from the LLS, these differences were not detectable. Additionally, LLS symptoms most important in classifying AMS+ were different than ESQ symptoms suggesting the questionnaires may not be interchangeable during this early time period. While our findings were based on a substantial number of data points (360), future research should (1) investigate larger data sets to see if the trees remain robust across multiple studies, (2) examine the concept that a different questionnaire specifically for NH may be more sensitive to early AMS and related symptoms, and (3) consider that the symptoms chosen to be queried may influence the definition, diagnosis, and characterization of AMS.

Ethical Approval

For the protection of human subjects, the investigators adhered to policies of applicable Federal Law CFR 46. Investigators adhered to AR 70-25 and USAMRMC Regulation 70-25 on the use of volunteers in research.

Consent

Human subjects participated in these studies after giving their free and informed consent.

Disclosure

The views, opinions, and/or findings contained in this publication are those of the authors and should not be construed as an official Department of the Army position, policy, or decision unless so designated by other documentation. Any citations of commercial organizations and trade names in this report do not constitute an official Department of the Army endorsement of approval of the products or services of the organizations.

Competing Interests

The authors have no professional relationships with companies or manufacturers who will benefit from the results of the present study.

Acknowledgments

The authors gratefully acknowledge the support of the DOD CDMRP program and grant W81-XWH1020199. They also thank the following individuals for their assistance: Andrea Gunn, Janet Staab, Chuck Fulco, Beth Beidleman, Ingrid Sills, Vincent Forte, Marie Grunbeck, Sean Andrew, Katylin Guerriere, and Myra Jones.

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Hindawi Publishing Corporation
BioMed Research International
Volume 2016, Article ID 1460892, 7 pages
http://dx.doi.org/10.1155/2016/1460892

Research Article

Myokines in Response to a Tournament Season among Young Tennis Players

K. Witek,[1] P. Żurek,[2] P. Zmijewski,[3] J. Jaworska,[4] P. Lipińska,[5] A. Dzedzej-Gmiat,[4] J. Antosiewicz,[6] and E. Ziemann[4]

[1]*Department of Biochemistry, Institute of Sport, Warsaw, Poland*
[2]*Faculty of Physical Culture, University School of Physical Education Poznan, Gorzów Wielkopolski, Poland*
[3]*Department of Physiology, Institute of Sport, Warsaw, Poland*
[4]*Department of Physiology and Pharmacology, Gdansk University of Physical Education and Sport, Gdansk, Poland*
[5]*Department of Biomechanics, Institute of Sport, Warsaw, Poland*
[6]*Department of Bioenergetics and Physiology of Exercise, Medical University of Gdansk, Gdansk, Poland*

Correspondence should be addressed to E. Ziemann; ziemann.ewa@gmail.com

Received 6 May 2016; Revised 23 July 2016; Accepted 2 August 2016

Academic Editor: David Bellar

The study investigated changes in myokines, heat shock proteins, and growth factors in highly ranked, young, male tennis players in response to physical workload during the competitive season and their potential correlations with match scores. Blood collections were carried out at the beginning, the midpoint, and the end of the tournament season. Data analysis revealed a significant increase in interleukin 6 and its inverse correlation with the number of lost games ($r = -0.45$; 90% CI −0.06 to 0.77). Neither the irisin nor BDNF level changed notably, yet delta changes of irisin across the season significantly correlated with the number of games won. The concentration of HSP27 recorded a small increase (31.2%; 90% CI 10.7 to 55.5, most likely). A negative correlation was noted between IGF-1 and HSP27 concentration at baseline (−0.70 very high; 90% CI −0.89 to −0.31, very likely). At the end of the season IGF-1 correlated positively with the number of games won ($r = 0.37$ moderate, 90% CI −0.16 to 0.73, likely) but negatively with the number of games lost ($r = -0.39$, 90% CI −0.14 to −0.74, likely). In conclusion our data indicated that Il-6, irisin, and growth factor IGF-1 may modify overall performance during a long lasting season, expressed in the amount of games won or lost.

1. Introduction

During a competitive season, professional tennis players experience mental and physical stress. Monitoring stress-inducing and manifesting factors is challenging; in research it is most often attempted during practice [1, 2] or a simulated tennis tournament [3]. Observations regarding the effects of the whole competitive season on the psychological and physical response are thus limited. The previous study revealed that during a tournament season most of the tennis players exhibited an elevated concentration of proinflammatory cytokines such as tumour necrosis factor (TNFα) or interleukin-1beta (IL-1β) [4, 5], which to some extent correlated inversely with the synthesis of heat shock proteins (HSP) [6]. Some of them, such as HSP27 and HSP70, were described as important for the repair and stabilization of stressed and damaged proteins [7]. Miller-Graziano et al. [8] stated that HSP27 belongs to a new group of "antidanger signals" that play a direct role in protecting against oxidative stress induced by exercise. Moreover, an elevated concentration of HSP70 is considered as a novel fatigue-signalling factor, sent from the immune system to the brain [9].

With regard to competitive sports, the focus of researchers and practitioners is on diagnosing and detecting signs of excessive mental and physical overload, associated with training sessions and competitions. It is particularly difficult or even impossible to measure physical workload among a group of tennis players, who train and plan their tournament seasons individually. In addition, monitoring of training loads in tennis is very demanding due to many variables that

need to be accounted for undefined duration of activity and rest, number of games, matches, and tournaments played and unpredictable weather conditions throughout. These conditions make it difficult to establish a one model of periodized training plan. Moreover, altogether these factors may disturb the proper balance between anabolic and catabolic processes, which is crucial for adaptation processes.

The defensive response depends on the synthesis of anabolic agents such as insulin growth factor IGF-1, proteins binding, and regulating its concentrations (IGF-BP) or myokines, which have an anti-inflammatory effect, regulate energy metabolism [10], and stimulate other tissues to synthesise proteins responsible for cognitive functions [11]. Similarly to other disciplines, in tennis the processes of perception and decision-making are very exhausting both mentally and in terms of energy expenditure [12].

Two particularly important myokines have been described: interleukin 6 (IL-6) and the newly discovered irisin [13]. IL-6 was originally classified as a prototypical proinflammatory cytokine, while later anti-inflammatory properties were also described [14, 15]. Irisin is secreted into the circulation following proteolytic cleavage from its cellular form, fibronectin-type III domain-containing 5 (FNDC5), in response to exercise [13]. Some data have shown that irisin is also secreted by adipose tissue [16, 17]. Its ability to increase the metabolic rate as well as effectiveness in enhancing the energy expenditure appears to be potentially therapeutic for obesity [18]. Irisin is also of interest to sport scientists. Daskalopoulou et al. [19] noted a correlation between irisin and intensity of exercise among young athletes. The findings of Nygaard et al. [20] underlined the relationships between single sessions of endurance and strength training and irisin concentration. At the same time, another study suggested that irisin may be a biomarker of muscle damage or act as a protective agent [21]. Furthermore, irisin has been shown to increase the expression of peroxisome proliferator-activated receptor γ coactivator-1α (PGC-1α), which plays an important role in the expression of brain-derived neurotrophic factor (BDNF) [11], which is relevant for brain health and development as well as cognitive functions [22]. In light of these developments, the aim of this study was to evaluate the influence of the whole tournament workload on blood myokine IL-6 and irisin in elite young tennis players. An additional purpose of the study was to reexamine the roles of BDNF, HSP27, and HSP70 in development of overreaching syndrome.

2. Methods

2.1. Overview. In this follow-up study, blood from young male tennis players was analysed on three occasions within the competitive season to evaluate the cumulative effects of match playing and its scores on selected biochemical indices. The study was conducted in the 2014 tournament season.

2.2. Subjects. Highly ranked, national-level, young, male tennis players (age 16 ± 2 years, singles national ranking 1–30) took part in the experiment. Overall, 12 players left the study at the beginning due to either high concentrations of proinflammatory markers ($n = 2$), injury ($n = 4$), fear of blood sampling/fear of weakening physical ability ($n = 2$), or cancelled participation in selected tournaments ($n = 4$), leaving 24 participants (Figure 1). Blood collection was carried out at the beginning of the tournament season (January), at its midpoint (May), and at the end (September). To examine the physical workload, all games of the participating players were recorded during this period using an online system provided by the Polish Tennis Association (PZA).

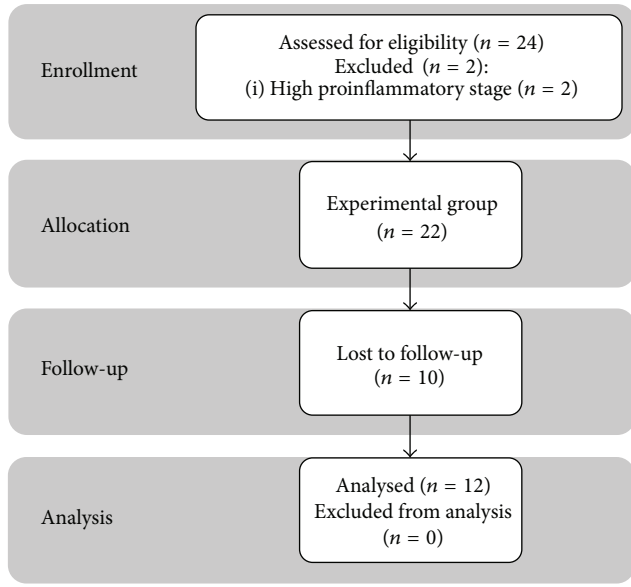

Figure 1: The schedule of examinations.

The examination was officially approved by the Bioethical Committee of the Regional Medical Society in Gdansk (KB-26/14) according to the Declaration of Helsinki. Participation had to be approved by written consent from the tennis players' parents.

2.3. Blood Sampling and Cytokine Analysis. Blood samples were taken from an antecubital vein into single-use containers with an anticoagulant ($EDTAK_2$). After collection, the samples were immediately stored at a temperature of 4°C. Within 20 minutes, they were centrifuged at 3000 g and 4°C for 10 min. Aliquots of the plasma were stored at −80°C. The blood was collected at rest, in the morning hours 7:30–8:00 a.m. in fasting state.

Serum (IL-6), interleukin-10 (IL-10), and TNFα concentrations were determined via enzyme immunoassay methods using commercial kits (R&D Systems, USA). The detection limits for TNFα, IL-6, and IL-10 were 0.039, 0.500, and 0.038 $pg \cdot mL^{-1}$, respectively. The average intra-assay CV was <8.0% for all cytokines.

Quantification of serum irisin was based on a competitive enzyme immunoassay and the assay kits purchased from Phoenix Pharmaceuticals Inc. (EK 067-16). The intra-assay coefficients of variability (CVs) and inter-assay CVs reported by the manufacturer were 4%–6% and 8%–10%, respectively.

Serum brain-derived neurotrophic factor was also detected using sandwich ELISA according to the

manufacturers' instructions (R&D Systems, USA; DY248). The detection limit for BDNF was 15 pg·mL^{-1}. Values are expressed as ng·mL^{-1}. Based on our previous experiences and Maffioletti's recommendation, 1-hour clotting duration for a correct serum BDNF dosage was applied [23].

Serum heat shock proteins HSP27 and HSP70 were evaluated using a Calbiochem ELISA kit (USA) and Stressgen kit (USA). Detection limits were 0.2 ng·mL^{-1}, and the intra-assay coefficient of variation for the kits was <5%.

Additionally, exercise-induced changes in plasma volume during the whole period of the investigation were calculated using the formula developed by van Beaumont et al. [24]. Thus, all myokines were recalculated according to changes in plasma volume using the formula proposed by Berthoin et al. [25].

2.4. Statistical Analysis. Measures related to blood parameters were analysed in a spreadsheet for a post-only crossover trial [26], and the effects were interpreted using magnitude-based inferences [27]. All data were log-transformed to reduce bias arising from the error nonuniformity. Means of the score changes, standard deviations of the score changes, and effects (differences in the changes of the means and their certainty limits) were backtransformed to percentage units. To improve the precision of estimates, the mean changes were adjusted to the log-transformed baseline mean. Magnitudes of the effects were also evaluated with the log-transformed data by standardizing with the standard deviation of the baseline values. Threshold values for assessing magnitudes of standardized effects were 0.20, 0.60, 1.2, and 2.0 for small, moderate, large, and very large, respectively. Uncertainty in each effect was expressed as a 90% confidence limit as well as a probability of the true effect being substantially positive (an increase) or negative (a decrease). These probabilities were used to make a qualitative, probabilistic, nonclinical inference about the true effect: if the probability of the effect being a substantial increase or a substantial decrease was >5% in both cases (equivalent of 90% confidence interval overlapping thresholds for a substantial increase and decrease), the effect was reported as unclear; otherwise, it was considered clear and assigned the relevant magnitude value, with the qualitative probability of the true effect being a substantial increase, substantial decrease, or a trivial difference (whichever outcome had the largest probability). The following scale for interpreting the probabilities was used: 25–75%, possible; 75–95%, likely; 95–99.5%, very likely; >99.5%, most likely. This study involved the assessment of substantial changes in nine measures. To maintain an overall error rate of <5% for declaring one or more changes to have opposite magnitudes (a substantial decrease instead of an increase, and vice versa), the effects were also evaluated as clear or unclear with a threshold of 5%/5 (1%), equivalent to consideration of overlap of substantial values with a 98% confidence interval (CI).

Relationships between changes in blood parameters across the tournament season against the number of performed games (all, won, and lost) were also calculated using Pearson correlation coefficients. Outcomes were expressed as values with 90% confidence intervals [28]. The usual scale for correlation coefficients (0.1, 0.3, 0.5, 0.7, and 0.9 for low, moderate, high, very high, and nearly perfect, resp.) was used.

3. Results

Obtained data of blood collections are presented in Table 1. Data show changes in the mean values of the effect induced by the workload applied during the tournament season and the magnitudes of the recorded shifts. The online system provided by Polish Tennis Association summarized all the games in this evaluated period. The average number of all games was 42 (±17) and of won and lost games was 26 (±15) and 16 (±5), respectively. The total number of matches included singles as well as doubles games. The more games the players won, the more they performed (0.97; 90% CI: 0.91 to 0.99). The physical workload experienced across the whole tournament season (training and tournaments) elicited a large and very large, clear increase in the IL-6 concentration (in the middle of the season and after the whole season, resp.). A moderate clear increase in anti-inflammatory interleukin (IL-10) was recorded but only in the middle of the season. Among HSP proteins a small clear and very likely increase was noted in HSP70 concentrations, whereas HSP27 elevated in smaller range (likely). All of these effects were still clear at the 98% CI level. The tournament season had no influence on proinflammatory level of TNFα. The moderate possible decrease in irisin concentration was noted in the middle of the season and trivial, but also possible decrease, at the end of the season. The other effects among measured blood parameters were only likely small and unclear.

Calculation of relationships between changes in blood parameters and number of lost games showed negative, likely moderate to high correlations in changes in IL-6 and in IGFBP-3 ($r = -0.45$, 90% CI −0.06 to 0.77 (Figure 2); −0.43; 90% CI −0.77 to 0.09, resp.), while a likely positive high correlation was observed for changes in BDNF (0.49; 90% CI: 0.0 to 0.8). Although the tournament season was not significantly associated with irisin concentration, its delta changes across the season significantly correlated with the number of won games (likely moderate 0.45; 90% CI: −0.06 to 0.78, Figure 3), and the determination factor equalled 0.20. Among anabolic indicators delta changes of IGFBP-3 inversely and highly corresponded to the number of played games (−0.53; 90% CI −0.81 to 0.04). At the end of the season the IGF-1 level correlated positively with the number of games won ($r = 0.37$ moderate, 90% CI −0.16 to 0.73, likely) but negatively with the number of games lost ($r = -0.39$, 90% CI −0.14 to 0.74, likely).

4. Discussion

The main finding of the study is that the physical workload during the whole tournament season led to an elevated concentration of myokine IL-6 and a slight decrease in irisin concentration. It is worth noting that the rate of changes of IL-6 concentrations is inversely correlated with the number of games lost. Previous studies reported that IL-6 increases

Table 1: The immunological response induced by physical workload during tournament season. Measures related to blood parameters at baseline and changes in the measures in the middle of season and at the end of the season in young tennis players ($n = 12$).

	Baseline mean ± SD	Middle season change		After season change	
		Mean; CI	Inference	Mean; CI	Inference
TNFα ($pg{\cdot}mL^{-1}$)	1.15 ± 0.44	−8.8%; −21.6 to 6.0%	Trivial↓*	0.2%; −10.9 to 12.7%	Unclear
IL-6 ($pg{\cdot}mL^{-1}$)	1.12 ± 0.57	92.5%; 64.1 to 126%	Large↑****	280%; 182 to 413%	Very large↑****
IL-10 ($pg{\cdot}mL^{-1}$)	0.58 ± 0.21	73%; 11.3 to 169%	Moderate↑**	38%; −24.4 to 152%	Small↑*
HSP 70 ($ng{\cdot}mL^{-1}$)	0.18 ± 0.16	36.8%; 9.6 to 70.8%	Small↑*	126%; 57.4 to 223%	Small↑***
HSP 27 ($ng{\cdot}mL^{-1}$)	13.6 ± 7.11	5.9%; −6.9 to 20.6%	Trivial↑*	31.2%; 12.6 to 52.8%	Small↑**
IGF 1 ($ng{\cdot}mL^{-1}$)	228 ± 68	5.2%; −5.3 to 16.8%	Trivial↑*	9.5%; −7.9 to 30.2%	Unclear
IGFBP-3 ($ng{\cdot}mL^{-1}$)	4137 ± 617	6.5%; 0.5 to 12.9%	Small↑**	5.2%; −1.4 to 12.0%	Small↑*
Irisin ($ng{\cdot}mL^{-1}$)	24.2 ± 22.5	−9.0%; −23 to 7.2%	Moderate↓*	−2.1%; −11.8 to 8.6%	Trivial↓*
BDNF ($ng{\cdot}mL^{-1}$)	50.9 ± 12.9	−14.7%; −38.5 to 18.2%	Unclear	−6.1%; −33.1 to 31.7%	Unclear

CI: 90% confidence interval.
↑: increase; ↓: decrease.
Likelihood that the true effect is substantial: *possible, **likely, ***very likely, and ****most likely.

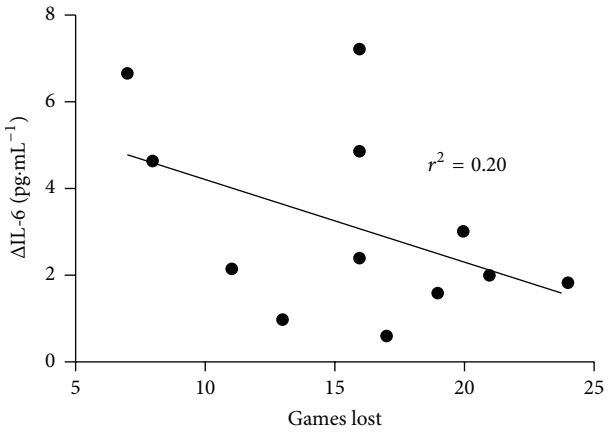

Figure 2: The relationship between delta changes in IL-6 concentration and number of lost games ($r = -0.45$, 90% CI −0.06 to 0.77, moderate, likely).

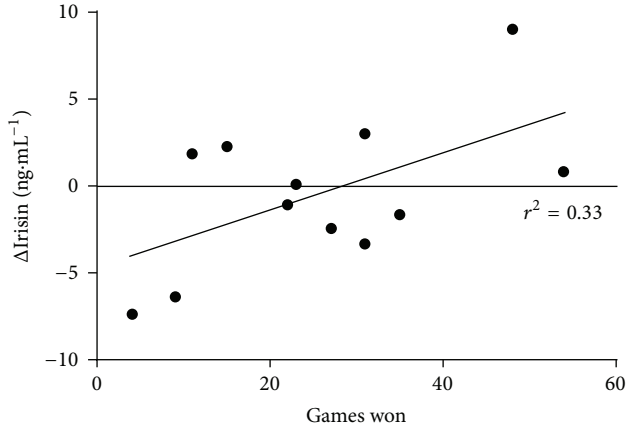

Figure 3: The correlation between delta changes in irisin concentration and number of won games ($r = 0.57$; 90% CI 0.01 to 0.83, high, very likely).

exponentially during a physical effort in relation to the intensity and duration of exercise, the mass of working muscles, and the individual's endurance capacity [29, 30]. Moreover, the biological role of IL-6 was described as an important energetic sensor, suggesting that muscle-liver crosstalk is mediated via IL-6 in regulating plasma glucose levels through endogenous glucose production during exercise [31]. In young adolescents a negative correlation of the amount of physical activity and plasma IL-6 concentration was found [32]. The significant shift of IL-6, which was observed at the end of the tournament season, indicates that those players who were characterized by greater changes in IL-6 concentration made fewer mistakes during the games. We can speculate that the rise of IL-6 can be treated as the anti-inflammatory response, which was supported by the increase of IL-10. Both allowed our players to avoid injuries or even an overreaching syndrome. Our results correspond with Halson's hypothesis, showing that reductions in IL-6 may lead to an altered metabolism of carbohydrate and fatty acids in the formation of ATP within skeletal muscle and induce the immune system dysfunction [33, 34]. Moreover, the latest paper by Wojewoda and coworkers revealed that

IL-6 could be involved in the regulation of a moderate-intensity training-induced enhancement of muscle oxidative phosphorylation activity in locomotors skeletal muscles [35]. These findings let us suggest that the tennis players characterized by the elevated blood level of IL-6 had been well adapted to the long lasting season workload and had achieved better scores in tournaments. Still, in our group enhanced synthesis of HSP70 at the end of the season was recorded. The obtained data confirm the previous observation [6] that a physical and mental workload leads to HSP70 production. The relationship between the rise of IL-6 and HSP70 was not significant but demonstrated that an increase of IL-6 inhibits HSP70 synthesis. The lack of statistical significance may be due to the small number of participants and should be verified in further studies.

One of the factors, which can influence obtained data, is the number of tournaments and games played by each player. For example, in our group of tennis players the total number of tournaments and games was lower than the number performed by the best players according to the International Tennis Federation. The best juniors played throughout season 91 (no. 1) and 90 (no. 2) singles and doubles matches. The average top 10 juniors played 81 games per season. The best player under 16 (no. 1) played 43 matches, but no. 2 played as many as 98 matches. The average top 10 played 58 matches per season. In our group the tennis players who played more won more games. On one hand, it seems to be logical that the more they played, the more experience they gained; but on the other hand, the more they played, the more physical work they performed and they could feel more exhausted. In training and competition it is demanding to maintain a balance between anabolic and catabolic response. Thus, we evaluated the influence of competitive season on IGF-1 and IGFBP-3. The latter belongs to the family of binding proteins which bind IGF-1 in the blood and in the extracellular matrix [36]. IGF-1 bound by IGFBP-3 cannot interact with a receptor, which inhibits its effect on gene expression [37]. At the same time, IGF-1 bound by IGFBP-3 is protected against prompt removal from the blood circulation; thus increase of its concentration may potentiate IGF-1 effects [38].

In our study we observed both proteins to have increased during the season; however, the increase of IGFBP-3 was much more pronounced, and it can be considered as an adaptive response. In addition, among our tennis players a significant, inverse correlation between delta change of IGFBP-3 and amount of lost games was observed. Previously, in endurance sports, a fatigue-dependent course was observed for IGF-1, but this kind of change was not observed as a cumulative effect of sports game training. It was proposed that because IGF-1 and IGFBP-3 are functionally connected and mainly represent the metabolic aspect of fatigue, a different kind of tennis match and training as compared to endurance training may therefore partly explain the smaller effect sizes of fatigue-induced changes [39]. Elevated concentrations of IGF-1 and IGFBP-3 were noted in the high level training group of young volleyball players after 18 months of intensive training compared to controls [40]. However, other patterns could also be observed. It was proposed that the state of a decrease in IGF-1 accompanied by an increase in IGF-BP3 could indicate a state of glucose austerity after depletion of carbohydrate stores due to endurance training [41]. In young boxers, IGF-1 and IGF-BP3 did not change significantly after a 5-week period of intense training but greatly increased after one week of tapering [42], suggesting an adaptive response. IGF-1 and IGF-1/cortisol ratio were found to be sensitive markers of training load and physical performance variations [42]. Moreover, in young individuals, a positive relationship was found between IGF-1 concentration and physical performance [43].

In our study the second myokine and irisin was considered as an important factor, which may not only regulate metabolism but also stimulate cognitive functions [11]. In contrast to our expectation, the effect of the tournament season caused a trivial decrease in the concentration of irisin and consequently BDNF level. Interestingly, a positive correlation was noted between the rate of change of irisin and number of won games. These data support the concept that irisin may be a link connecting function of skeletal muscle and brain. On the other hand, no changes were recorded in BDNF concentration. It has been also shown that serum BDNF levels reflect the BDNF concentration in the brain; therefore, measurements of the serum BDNF concentration can be used to monitor its changes in the brain [44]. In another study, both acute aerobic and anaerobic activity elevated serum BDNF in athletes. It was suggested that long-term habitual exercise is associated with lower peripheral BDNF and better intermediate memory [45]. However, acute forms of intensive activity, either aerobic or anaerobic, are able to elevate serum BDNF level in both sedentary persons and athletes [45]. It was also reported that endurance training of moderate intensity increases both basal and end-exercise BDNF levels in young healthy men [46]. These results suggest a possible relationship between irisin and cognitive function among our tennis players. One of the factors which can modulate BDNF synthesis is the proinflammatory cytokine TNFα [47]. It is also known that other factors, which induce inflammation, contribute to reducing the BDNF concentration [48, 49]. Also, some types of athletic activities like heading a ball could increase the BDNF concentration in the blood, which is related to a microtrauma of the brain tissue [47], but this type of movement act is not typical for a tennis activity and has a rather minor contribution. In our tennis players we did not observe any significant rise in TNFα, neither in the middle, nor at the end of the tournament season. Obtained serum BDNF concentrations in our group exhibited elevated values in comparison to the recommended ones [48].

5. Conclusion

To authors knowledge this is one of the first study presenting changes of broad biochemical and immunological indices within competitive season in tennis players. Despite being partially limited by the small number of subjects and lack of monitoring of training workload of each player, the report provides selected reference data.

Present data demonstrating that myokines IL-6 and irisin and IGF-1 and IGFBP-3 could be useful markers in monitoring tennis workload and exercise adaptations. The observed

changes indicate these factors contribute to a defence mechanism and have an impact on the cognitive functions, which enables players to make better, more strategic decisions during game.

Competing Interests

The authors declare that they have no competing interests.

Acknowledgments

This study was funded by a grant from the Ministry of Sport and Tourism of the Republic of Poland 2014.031/40/BP/DWM. The authors would like to thank the Polish Tennis Association for support and help in conducting and organising the research. Moreover, they would like to thank all the tennis players who participated in the study and their parents for forbearance.

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Hindawi Publishing Corporation
BioMed Research International
Volume 2016, Article ID 5387258, 12 pages
http://dx.doi.org/10.1155/2016/5387258

Research Article

Effects of 8-Week Hatha Yoga Training on Metabolic and Inflammatory Markers in Healthy, Female Chinese Subjects: A Randomized Clinical Trial

Neng Chen,[1] Xianghou Xia,[2] Liqiang Qin,[1] Li Luo,[3] Shufen Han,[1] Guiping Wang,[4] Ru Zhang,[1] and Zhongxiao Wan[1,5]

[1]*Department of Nutrition and Food Hygiene, School of Public Health, Soochow University, 199 Renai Road, Suzhou 215123, China*
[2]*Department of Breast Surgery, Zhejiang Cancer Hospital, Hangzhou, Zhejiang 310022, China*
[3]*School of Physical Education and Sports Science, Soochow University, Suzhou 215006, China*
[4]*Laboratory Animal Center, Soochow University, 199 Renai Road, Suzhou 215123, China*
[5]*Jiangsu Key Laboratory of Preventive and Translational Medicine for Geriatric Disease, Soochow University, 199 Renai Road, Suzhou 215123, China*

Correspondence should be addressed to Zhongxiao Wan; zhxwan@suda.edu.cn

Received 23 January 2016; Accepted 30 June 2016

Academic Editor: Edward J. Ryan

We aimed to determine the effects of an 8 wk Hatha yoga training on blood glucose, insulin, lipid profiles, endothelial microparticles (EMPs), and inflammatory status in healthy, lean, and female Chinese subjects. A total of 30 healthy, female Chinese subjects were recruited and randomized into control or yoga practice group. The yoga practice included 8 wks of yoga practice (2 times/wk) for a total of 16 times. Fasting blood samples were collected before and after yoga training. Plasma was isolated for the measurement of lipid profiles, glucose, insulin, EMPs, and inflammatory cytokines. Whole blood was cultured *ex vivo* and stimulated with lipopolysaccharide (LPS) and Pam3Cys-SK4. Peripheral blood mononuclear cells (PBMCs) were isolated for the measurement of TLR2 and TLR4 protein expression. Yoga practice significantly reduced plasma cholesterol, LDL-cholesterol, insulin levels, and CD31+/CD42b− EMPs. Cultured whole blood from the yoga group has reduced proinflammatory cytokines secretion both at unstimulated condition and when stimulated with Pam3Cys-SK4; this might be associated with reduced TLR2 protein expression in PBMCs after yoga training. Hatha yoga practice in healthy Chinese female subjects could improve hallmarks related to MetS; thus it can be considered as an ancillary intervention in the primary MetS prevention for the healthy population. This trial is registered with ChiCTR-IOR-14005747.

1. Introduction

Yoga is a mind-body therapy that has become increasingly popular worldwide. Accumulating evidence suggests that yoga meditation could improve risk factors associated with metabolic syndrome (MetS) such as obesity, disordered lipid profile [1], and insulin resistance [2–4]. However, most of these studies are conducted in clinical populations [1–4] and there are surprisingly few studies examining how yoga training affects MetS' related risk factors in healthy subjects [5, 6]. In regard to this, Manjunatha et al. [5] reported that 5 days of yoga asanas increased the sensitivity of the β cells to the glucose signal in healthy subjects. Bhattacharya et al. [6] found that yoga practice can improve the antioxidant status of the healthy individual.

Endothelial microparticles (EMPs) are complex vesicular structures shed from endothelial cells in response to stimuli such as inflammatory activation [7]. They are now considered as novel biomarkers of endothelial activation and damage that are increased in overweight/obese individuals at risk for MetS [8, 9]. Evidence suggests that EMPs change with alterations in physical activity (PA) [10–12]. For example, reduced daily PA by taking <5,000 steps/day with a total of 5 days resulted in elevated CD31+/CD42b− EMPs in recreationally

active men [12]. Similarly, enforced physical inactivity by subjecting healthy men to 7 days of dry immersion also led to increased circulating CD31+/CD41− EMPs [10]. In contrast, 6 months of supervised aerobic exercise training with moderate intensity could improve circulating EMPs levels as evidenced by decreased CD31+/CD42− EMPs in sedentary African American adults [11]. As yoga practice altered the blood flow velocity and consequently shear stress at the vascular wall [13], presumably it could affect EMPs. However, currently, there is no evidence whether yoga practice could affect EMPs, especially in Chinese subjects.

Inflammation is one of the key mechanisms involved in the pathogenesis of MetS [14]. Presently evidence examining effects of yoga on inflammatory processes is limited. Existing evidence suggests that yoga could positively affect circulating inflammatory markers in heart failure patients [15, 16], breast cancer survivors [17, 18], and patients with chronic inflammatory diseases and overweight/obese subjects [19]. Meanwhile, mind-body interventions that include some elements of yoga can reduce inflammatory signaling through NF-κB pathway [17, 20, 21]. Toll-like receptors (TLRs), especially TLR2 and TLR4, play critical roles in innate immunity and may be involved in the link between physical activity, inflammation, and MetS [22–24]. However, it remains unclear whether yoga practice could affect circulating TLR2 and/or TLR4 response in healthy subjects.

Hatha yoga is the most commonly practiced worldwide. The key components of Hatha yoga are stretching exercises and physical postures, breath control, and concentration and thinking techniques designed to promote the well-being of the subjects both physically and emotionally [25]. With the above points in mind, the primary aim of the present study is to determine the effects of an 8 wk Hatha yoga practice on metabolic markers including blood glucose, insulin, lipid profiles, blood pressure, and EMPs in healthy, lean, and female Chinese subjects; the secondary aim is to determine the effects of Hatha yoga practice on inflammatory markers in the above subjects via measuring circulating cytokine levels, LPS, and Pam3Cys-SK4 (Pam) stimulated cytokines secretion in whole blood culture *ex vivo*, as well as TLR2 and TLR4 protein expression in PBMCs.

2. Materials and Methods

2.1. Materials. RPMI-1640, LPS (cat. number L6529-1) and 900 nm Latex beads carboxylate modified polyester (CLB9) were from Sigma (MO, USA). A custom human Adipokine Panel 2 (cat. number HADK2MAG-61K) containing primary and detection antibodies for interleukin- (IL-) 6, IL-8, IL-1β, monocyte chemoattractant protein- (MCP-) 1, tumor necrosis factor- (TNF-) α, and insulin was purchased from Merck Millipore (MA, USA). Pam (cat. number tlrl-pms) was from InvivoGen (CA, USA). Human IL-6 (cat. number DY206), IL-1β (cat. number DY201), and TNF-α (cat. number DY210) ELISA kit (DuoSet) was from R&D Systems (NE, USA). Antibodies against CD42b-PE (cat. number 555473), CD31-FITC (cat. number 555445), and CD62E-APC (cat. number 551144) were from BD Biosciences (NJ, USA). An antibody against TLR2 (cat. number 12276) was from Cell Signaling Technologies (MA, USA). TLR4 antibody (cat. number MAB1478) was from R&D Systems (NE, USA). All other chemicals were purchased from Sigma (MO, USA) unless otherwise noted.

2.2. Trial Design and Changes after Trial Commencement. This investigation reports a single-arm parallel-randomized controlled trial comparing the effects of 8 weeks of yoga intervention on metabolic and inflammatory markers in healthy female subjects. Ethical approval was obtained from the Human Research and Ethical Committee of the Soochow University and all participants provided signed informed consent. All methods were performed following the approved guidelines and regulations. This trial was registered in the Chinese Clinical Trial Registry with the number ChiCTR-IOR-14005747 on December 27, 2014. No changes to the methodology occurred following trial commencement. The data were reported according to the CONSORT statement [26].

2.3. Participants, Eligibility Criteria, and Settings. This study was conducted at School of Public Health, Soochow University, Jiangsu Province, China. Participants were recruited from the Campus of Soochow University via poster advertisement. The study inclusion criteria included age 18–25 years old; BMI > 18.5 and <23.9 kg/m^2; the blood glucose, triacylglycerol, cholesterol, HDL-C, LDL-C, systolic blood pressure (SBP), and diastolic blood pressure (DBP) being within the normal ranges; and self-reported regular menstrual cycles (i.e., cycle 24–36 days long and at least 10 cycles in the previous 12 months). The exclusion criteria included subjects having history for using of pharmacologic contraceptives (past 6 months) and history of breast cancer, heart diseases, diabetes mellitus, or other serious medical conditions and subjects suffering from musculoskeletal conditions that would prevent participation in a yoga training.

2.4. Interventions. A total of 30 female subjects were recruited and randomized into control or yoga practice group. Participants in the yoga group were then asked to attend supervised Hatha yoga sessions 2 times per week over the 8 wks of the study. Yoga classes were offered on Monday and Thursday every week (from 6 p.m. to 7 p.m.). Each class has a total of 60 minutes and had the following components: breathing exercise (6 mins); loosening exercise (i.e., corn tree pose) (10 mins); standing poses (i.e., warrior pose and mountain pose) (8 mins); supine poses (i.e., bridge pose and dolphin plank pose) (8 mins); prone poses (i.e., hare pose and locust pose) (8 mins); sitting poses (i.e., staff pose and hero pose) (8 mins); relaxation/corpse pose (6 mins); and seated meditation (6 mins). Approximately 32 minutes is spent in active poses. The classes were held in a yoga training room and taught by a registered, specialized yoga instructor. The yoga practice was specifically designed for this study; however the yoga classes were not observed by study staff. Subjects were also instructed to maintain their usual physical activity and dietary habits for the study.

2.5. Primary and Secondary Outcomes. On day 1 of the study and 2 days after the whole yoga practice, subjects reported to the laboratory after an overnight fast; a baseline and a final blood sample (10 mL), respectively, were obtained by venipuncture from an antecubital vein and collected into EDTA tubes. Blood (9 mL) was centrifuged at 1500 g for 10 mins at 4°C and plasma was immediately frozen at −80°C for subsequent batch analyses of plasma cytokines, clinical biomarkers (i.e., insulin, glucose, triacylglycerol (TG), HDL-cholesterol, LDL-cholesterol, and total cholesterol), and endothelial microparticles. About 1 mL blood was utilized for whole blood culture. The height, body weight, SBP, and DBP of the subjects were measured by trained research assistants following standardized procedures using calibrated equipment.

The primary outcome measure for this trial was plasma insulin level, while secondary outcomes were (1) other clinical biomarkers (i.e., glucose, TG, HDL-cholesterol, LDL-cholesterol, and total cholesterol); (2) EMPs; and (3) plasma cytokines and cytokines from culture whole blood *ex vivo*. There were no changes to outcomes following trial commencement.

2.6. Sample Size Calculation. The sample size was based on (1) published findings from other research groups who have reported the beneficial effects of yoga with similar sample size [5, 6, 15] and (2) calculations assuming two-tailed $\alpha = 0.05$ and 1-β = 90% to detect a 10% difference for the plasma insulin levels, which is the primary outcome of the present study.

2.7. Randomization and Blinding. Following recruitment randomization was carried out via computer-generated random numbers with unrestricted equal participant allocation (1 : 1) by one research investigator, who is independent of the yoga intervention and data analysis. Participants were not blinded to the study.

2.8. Plasma Clinical Metabolic Biomarkers Measurement. Clinical biomarkers including glucose, TG, HDL-cholesterol, LDL-cholesterol, and total cholesterol were measured on an automatic analyzer (Hitachi 7600, Tokyo, Japan). The homeostasis model assessment of insulin resistance (HOMA-IR) was calculated using the following equation: HOMA-IR = fasting insulin (FIns, μIU/mL) × fasting blood glucose (FBG, mmol/L)/22.5.

2.9. Plasma Cytokines and Insulin Measurement. Plasma cytokines including IL-6, IL-8, IL-1β, MCP-1, TNF-α, and plasma insulin were measured from EDTA plasma using Luminex® technology according to the kit manufacturer's instructions. The detection limits for IL-6, IL-8, IL-1β, MCP-1, TNF-α, and insulin were 0.2, 0.3, 0.4, 1.2, 0.3, and 3.8 pg/mL, respectively. Plasma IL-6 and IL-1β levels were below the detection limit of the assay in our study. The average CV for duplicates in the assay is <6%.

2.10. Endothelial Microparticles (EMPs) Measurement. Circulating EMPs were measured in platelet-poor plasma by flow cytometry following the method of Jenkins et al. [27]. In brief, frozen plasma samples were thawed at room temperature for 20 minutes and centrifuged at 1500 g for 15 minutes. The top two-thirds of plasma were then further centrifuged at 1500 g for another 15 minutes to obtain platelet-poor plasma. The top 100 μL of platelet-poor plasma was then incubated with fluorochrome labeled antibodies specific for PE-CD42b, FITC-CD31, and APC-CD62E for 20 minutes in the dark at 4°C. Samples were then fixed with 93 μL of 2% paraformaldehyde and diluted up to 500 μL with sterile, 0.2 μM filtered PBS and analyzed on a FC500 Beckman Coulter (CA, USA). A microparticle size gate was determined using 900 nm Latex beads carboxylate modified polyester. Unstained and fluorescence minus one controls were used to differentiate between true events and background/debris. EMPs were identified as CD62E+ and CD31+/CD42b− events within the microparticle size gate.

2.11. Whole Blood Culture. Whole blood was diluted 1 : 10 with serum-free RPMI-1640 medium (penicillin 100 U/mL, streptomycin 100 μg/mL) (i.e., 540 μL whole blood diluted in 4.86 mL RPMI-1640 medium), plated in duplicate on 24-well plates at a final volume of 600 μL, and cultured at 37°C in a humidified incubator (5% CO_2) as described by Wan et al. [28]. Samples were stimulated with the TLR4 agonist LPS (1, 10 ng/mL) and TLR2 agonist Pam3Cys-SK4 [29] (1, 10 ng/mL) and supernatants were harvested after 24 h via centrifuge at 2000 g for 15 min at 4°C. Samples were then stored at −80°C before batch analysis of TNF-α, IL-6, and IL-1β via ELISA according to the manufacturer's instructions. Biological replicates were analyzed, with the average coefficient of variation (CV) for each cytokine being <5%.

2.12. PBMCs Isolation. PBMCs were isolated by gradient density centrifugation of peripheral blood using Ficoll-Paque Plus as described previously by our laboratory [28]. Briefly, 5 mL of blood was layered onto 5 mL of Ficoll-Paque Plus in a sterile 15 mL tube and was centrifuged for 15 min at 800 g and at 20°C. The layer of PBMCs was recovered and washed three times with sterile PBS for 10 min at 250 g at room temperature. Isolated PBMCs were then stored at −80°C until further protein expression analysis by western blotting.

2.13. Western Blotting. Proteins from isolated PBMCs were extracted. The protein expression of TLR2 and TLR4 was determined by western blotting following the methods published by our laboratory previously [30]. Signals were visualized using Immobilon western chemiluminescent HRP substrate and bands were quantified by densitometry. Beta actin was used as an internal control.

2.14. Statistical Analysis. All data are presented as mean ± standard error of the mean (SEM). Statistical analyses were performed with SPSS version 15.0 for Windows (IL, USA). Data were analyzed for normality and homogeneity before statistical test. Two-way ANOVA was utilized for comparisons between groups. Tukey's Honestly Significant Difference (HSD) was applied for post hoc comparisons. Statistical significance was set at $p < 0.05$.

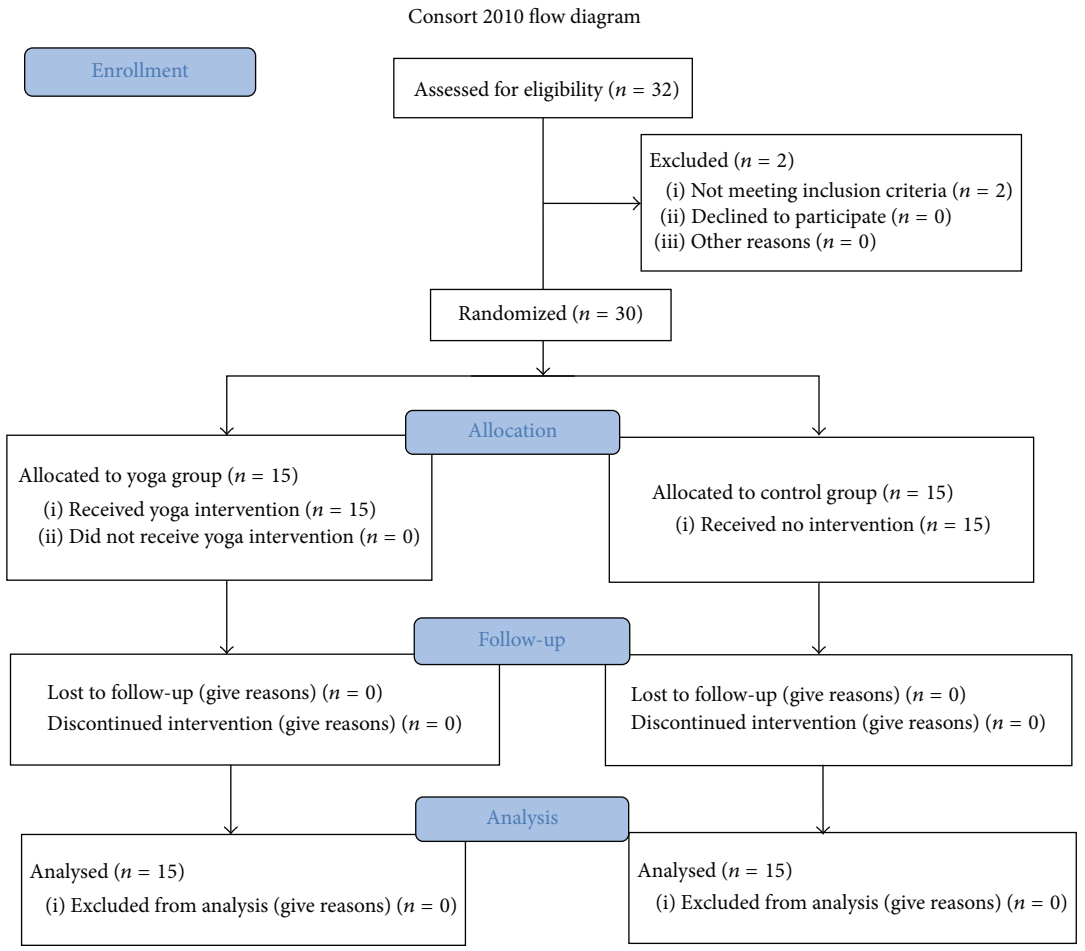

Figure 1: CONSORT flowchart.

3. Results

3.1. Participants' Flow and Participation Rate. The CONSORT flowchart of subject recruitment and intervention was shown in Figure 1. From March 2015 to June 2015, all recruited subjects completed the whole yoga practice with no dropout. There were no harmful effects observed by the yoga practice.

3.2. Yoga Practice Decreased Plasma Insulin, Total, and LDL-Cholesterol Level. A total of 8 wks yoga practice resulted in significant reduction in plasma insulin, total cholesterol, and LDL-C levels compared to preyoga practice; meanwhile, HOMA-IR from yoga group is reduced compared to both yoga groups at baseline level and control group after intervention, while there is no difference for glucose, TG, HDL-C, SBP, DBP, body weight, and BMI before and after yoga practice between groups (Table 1).

3.3. Yoga Practice Reduced Circulating CD31+/CD42b− EMPs. As shown in Figure 2, there was a significant reduction in circulating CD31+/CD42b− EMPs after yoga intervention compared to yoga group at baseline level and control group (Figures 2(a) and 2(b)), while yoga practice had no effect on CD62E+ EMPs (Figures 2(c) and 2(d)).

3.4. No Effect of Yoga Practice on Circulating Proinflammatory Cytokines. As shown in Table 2, there were no significant effects of yoga practice on levels of plasma proinflammatory cytokines (IL-8, MCP-1, and TNF-α) measured in the fasted state.

3.5. Yoga Practice Resulted in Decreased Proinflammatory Cytokine Response. At baseline level, yoga group demonstrated elevated IL-6 secretion in supernatant from cultured whole blood at unstimulated condition (Figure 3(a)). Yoga group had reduced secretion of IL-6, TNF-α, and IL-1β levels after yoga training (Figure 3). Furthermore, when cultured blood was challenged with Pam at both 1 ng/mL and 10 ng/mL, a well-known agonist of TLR-2 receptor [29], yoga practice group also demonstrated damped cytokines secretion including IL-6, TNF-α, and IL-1β levels compared to preyoga condition and control group (Figure 4). Meanwhile, at baseline level, yoga group has reduced TNF-α secretion compared to control group when stimulated with LPS (at both 1 ng/mL and 10 ng/mL); this trend was maintained after yoga training (Figure 5(b)). There is no difference for IL-6 and IL-1β secretion when stimulated with LPS (Figures 5(a) and 5(c)).

Table 1: Comparison of metabolic characteristics between groups before and after yoga intervention.

	Control		Yoga	
	Pre	Post	Pre	Post
Insulin (mIU)	6.17 ± 0.60	5.55 ± 0.75	6.58 ± 0.98	4.06 ± 0.87*
Glucose (mM)	4.59 ± 0.07	4.51 ± 0.08	4.59 ± 0.13	4.48 ± 0.1
HOMA-IR	1.26 ± 0.12	1.13 ± 0.17	1.36 ± 0.21	0.75 ± 0.18*,#
TG (mM)	0.60 ± 0.06	0.60 ± 0.04	0.66 ± 0.03	0.68 ± 0.09
Cholesterol (mM)	3.90 ± 0.18	3.64 ± 0.15	4.13 ± 0.12	3.75 ± 0.15*
LDL-C (mM)	1.93 ± 0.15	1.76 ± 0.13	2.14 ± 0.11	1.81 ± 0.13*
HDL-C (mM)	1.69 ± 0.07	1.68 ± 0.05	1.67 ± 0.05	1.58 ± 0.05
SBP (mmHg)	108.0 ± 2.7	105.9 ± 1.5	106.8 ± 2.1	102.5 ± 2.3
DBP (mmHg)	76.15 ± 1.8	72.62 ± 1.8	74.77 ± 2.4	71.83 ± 2.00
Body weight (kg)	54.08 ± 1.65	53.81 ± 1.68	53.35 ± 1.53	52.71 ± 1.57
BMI (kg/m^2)	20.68 ± 0.46	20.18 ± 0.46	20.55 ± 0.52	20.49 ± 0.52

TG: triacylglycerol; LDL-C: low density lipoprotein-cholesterol; HDL-C: high density lipoprotein-cholesterol; SBP: systolic blood pressure; DBP: diastolic blood pressure; and BMI: body mass index
Data are expressed as mean ± SEM. *Compared with preintervention baseline level; #compared with control group after intervention.

Table 2: Plasma cytokines measured in the fasted state before and after yoga intervention.

	Control		Yoga	
	Pre	Post	Pre	Post
IL-8 (pg/mL)	6.17 ± 0.61	5.13 ± 0.52	5.64 ± 0.56	5.03 ± 0.34
MCP-1 (pg/mL)	159.70 ± 20.86	148.95 ± 20.00	145.71 ± 19.95	149.52 ± 14.44
TNF-α (pg/mL)	2.15 ± 0.30	2.36 ± 0.38	1.66 ± 0.19	1.96 ± 0.30

Data are expressed as mean ± SEM.

3.6. Yoga Practice Resulted in Decreased TLR2 Protein Expression in PBMCs. As shown in Figure 6, there is no difference for TLR2 protein expression between groups at baseline level; yoga practice resulted in significant reduction in TLR2 protein expression in PBMCs, while there is no difference for TLR4 protein expression between groups before and after yoga practice.

4. Discussion

The main findings of the present study are that (1) 8 wks of Hatha yoga practice in healthy Chinese female subjects reduced plasma insulin, cholesterol levels, and circulating CD31+/CD42b− EMPs and that (2) cultured whole blood from yoga practice group had reduced proinflammatory cytokines secretion at unstimulated condition, as well as when stimulated with a TLR2 agonist, and this might be associated with reduced TLR2 protein expression after yoga training.

The most significant risk factors for MetS include dyslipidemia, hypertension, and physical inactivity [31]. Yoga practice improved lipid profiles in clinical patients with cardiovascular diseases [32, 33] and hypertension [34]. In particular, Bijlani et al. [34] reported that the TG-lowering effects of yoga were more prominent in subjects with hypercholesterolemia [34]. Therefore, when assessing yoga's effects on improving lipid profiles, it is important to consider participants' health conditions. Our present study confirmed that, in healthy, female Chinese subjects, 8 wks of Hatha yoga practice (2 times/wk) could reduce total cholesterol and LDL-C level, indicating that Hatha yoga practice is an effective way for reducing risk factors associated with disordered lipid profiles even in healthy subjects. Randomized trials [35] and meta-analyses [36] have consistently demonstrated a modest but consistent reduction in blood pressure following yoga practice. However, we observed no alterations in SBP and DBP after 8 wks of yoga practice. This might be related to multiple factors. First, different yoga practice type, length, and frequency might affect its effects on blood pressure. Second, the subjects in our present study are healthy; thus it might be hard to observe reductions in blood pressure.

Yoga has been increasingly accepted as a cost-effective therapeutic strategy for T2DM patients [2, 37]. Evidence in regard to how yoga practice affects plasma insulin level remains inconsistent. Hunter et al. [13] reported that Bikram yoga, which is one of the most popular forms of hot yoga, resulted in reduction in plasma insulin and HOMA-IR only in older adults (53 ± 2 yrs). Vizcaino [38] demonstrated that 6 wks of Hatha yoga (3 times/wk) has no effect on fasting insulin level in patients with T2DM. In contrast, Manjunatha et al. [5] reported that yoga practice reduced serum insulin level in healthy subjects, while the majority of them were male. Our present study further confirmed that in healthy female subjects 8 wks of Hatha yoga could significantly reduce plasma insulin level and consequently HOMA-IR.

Elevation of EMPs is rapidly being accepted as an alternate surrogate marker of CVDs and endothelial function

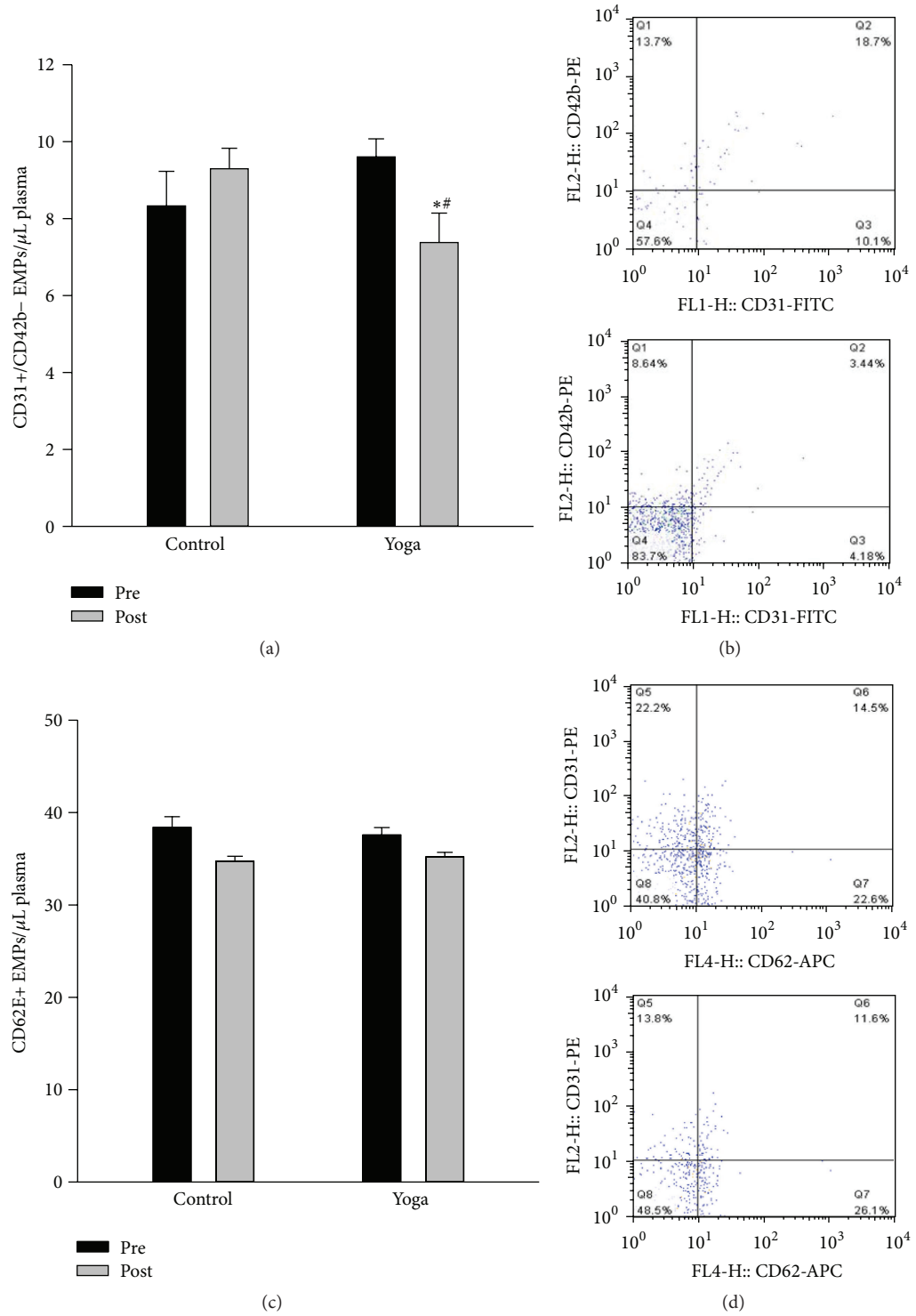

Figure 2: Yoga practice reduced circulating CD31+/CD42b− EMPs levels. Circulating EMPs were measured in platelet-poor plasma by flow cytometry with fluorochrome labeled antibodies specific for PE-CD42b, FITC-CD31, and APC-CD62E. EMPs were identified as CD62E+ and CD31+/CD42b− events with a diameter <1 μM. (a) Fasting CD31+/CD42b− EMPs were reduced postyoga *practice* compared to preyoga training condition. (b) Representative fluorescence-activated cell sorter dot plots of CD31+/CD42b− of a subjects before (top) and after (bottom) yoga *practice*. (c) No difference for CD62E+ EMPs between groups. (d) Representative fluorescence-activated cell sorter dot plots of CD62E+ of a subjects before (top) and after (bottom) yoga *practice*. Data are presented as mean + SEM ($N = 15$). $^{*}p < 0.05$ versus preyoga training condition within the same group in (a); $^{\#}p < 0.05$ versus control group at baseline level.

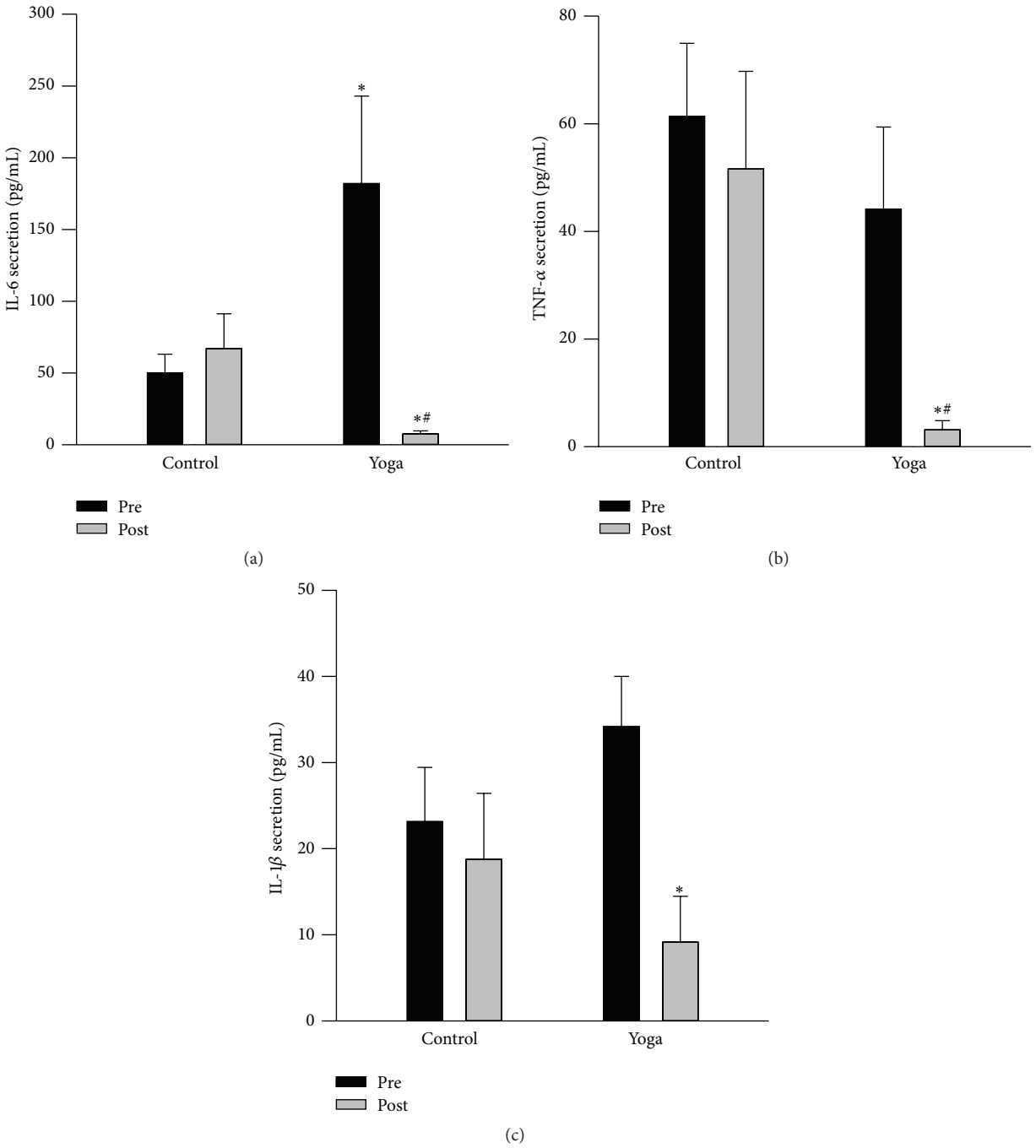

Figure 3: Reduced secretion of IL-6, TNF-α, and IL-1β from cultured whole blood *ex vivo* after yoga training. Whole blood was collected at baseline and after yoga; then blood was diluted and cultured at 24-well plates under identical culture conditions. Supernatants were centrifuged and collected at 24 hr for the measurement of IL-6, TNF-α, and IL-1β secretion via ELISA. There is significant reduction of IL-6 (a), TNF-α (b), and IL-1β (c) secretion after yoga compared to preyoga condition. Data are presented as mean + SEM ($N = 15$). $^{*}p < 0.05$ versus preyoga training condition within the same treatment; $^{\#}p < 0.05$ versus control group at baseline level.

[39]. CD62E+ EMPs generally reflect endothelial activation or inflammation whereas CD31+/CD42b− EMPs are released upon endothelial cell apoptosis [7]. Recent evidence has confirmed that moderate-intensity endurance training could reduce circulating EMP levels [11, 40, 41]. In contrast, physical inactivity via reducing daily PA [12] or subjecting subjects to 7 days of dry water immersion [10] is associated with increased concentrations of CD31+/CD42b− EMPs and CD31+/CD41− EMPs, respectively. Our study is the very first to reveal that 8 wks of Hatha yoga could significantly reduce plasma CD31+/CD42b− EMPs in healthy subjects. High concentrations of EMPs are associated with a proinflammatory

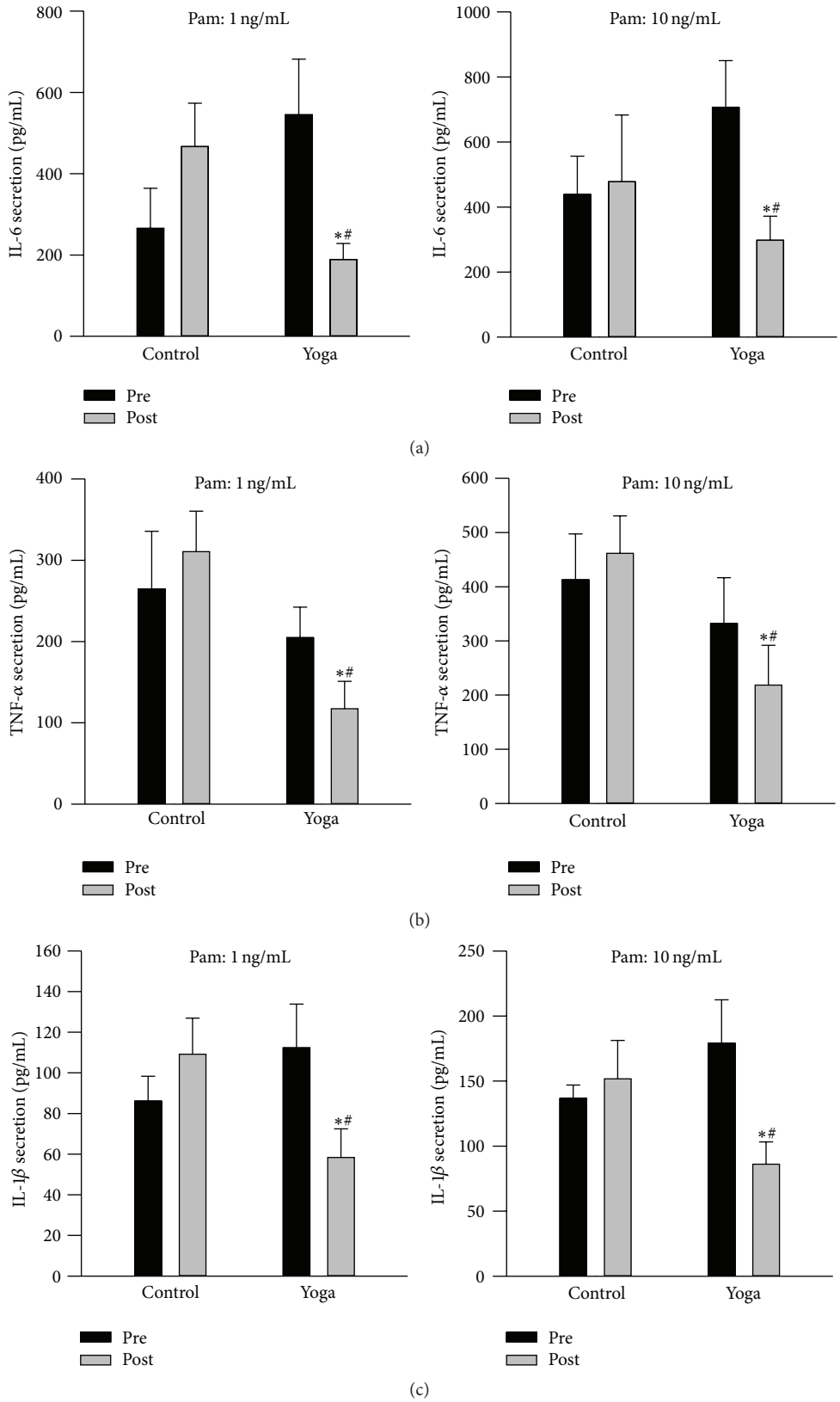

Figure 4: Attenuated Pam3Cys-SK4 (1 and 10 ng/mL) induced IL-6, TNF-α, and IL-1β secretion from *ex vivo* whole blood cultures after yoga practice. Whole blood was collected at baseline and after yoga; then blood was diluted, cultured at 24-well plates, and stimulated with Pam under identical culture conditions. Supernatants were centrifuged and collected at 24 hr for the measurement of IL-6, TNF-α, and IL-1β secretion via ELISA. Yoga training led to blunted IL-6 (a), TNF-α (b), and IL-1β (c) secretion upon Pam stimulation at both 1 and 10 ng/mL. Data are presented as mean + SEM ($N = 15$). $^{*}p < 0.05$ versus preyoga *practice* condition within the same treatment; $^{\#}p < 0.05$ versus control group at baseline level.

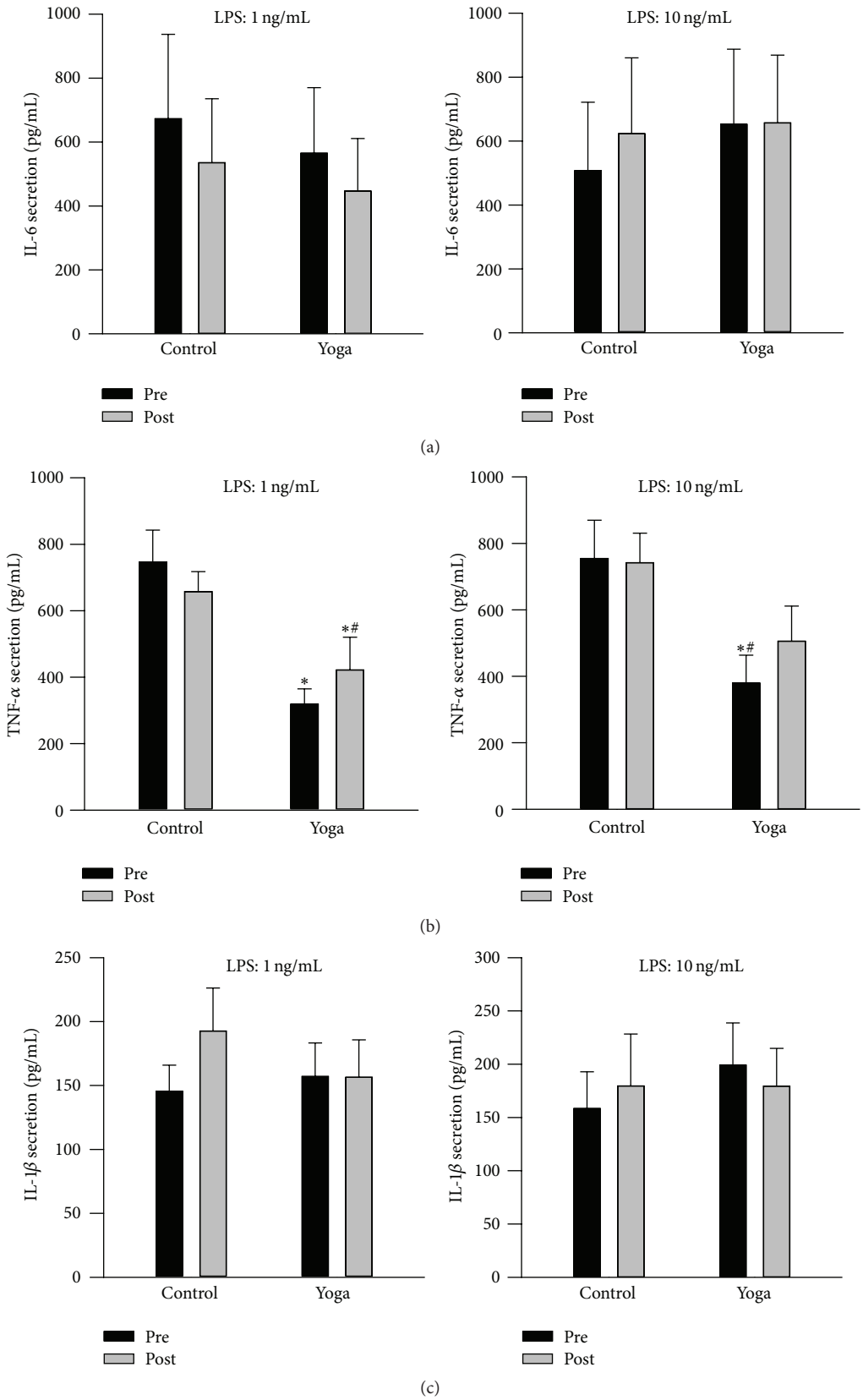

Figure 5: Reduction in TNF-α secretion from yoga group compared to control group at baseline and when stimulated with LPS. IL-6 (a), TNF-α (b), and IL-1β (c) secretion from groups. $^{*}p < 0.05$ versus control group at baseline level; $^{\#}p < 0.05$ versus control group postyoga practice condition.

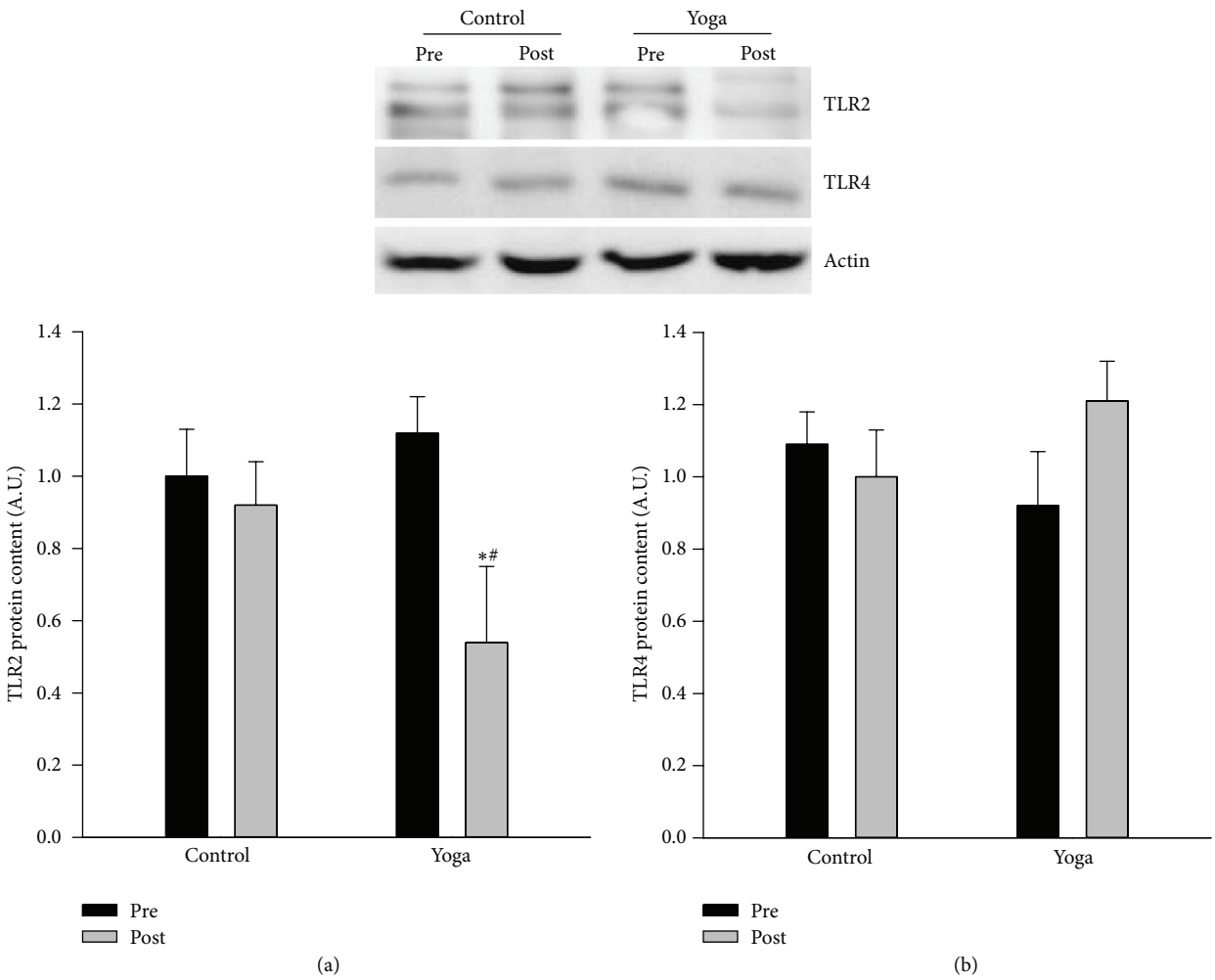

Figure 6: Reduction in TLR2 protein expression from PBMCs after yoga practice. PBMCs were isolated at baseline level and after yoga training, and the protein expression of TLR2 and TLR4 was measured via western blotting. Yoga practice resulted in reduction in TLR2 protein expression with no effect on TLR4. Western blotting images are given at the top of the quantified data. $^{*}p < 0.05$ versus preyoga training group; $^{\#}p < 0.05$ versus control group at baseline level.

and antiangiogenic status in the vascular system [42]; thus reduction of CD31+/CD42b− EMPs after yoga suggested that yoga might improve vascular function via affecting EMPs levels. Furthermore, Jenkins et al. [27] reported that an acute reduction in shear stress *via* disturbed blood flow increased local concentrations of CD31+/CD42b− and CD62E+ EMPs in the human forearm. Our result could also suggest that, unlike pathological stress, physiological stress like yoga may decrease EMPs release. This might be one of the mechanisms through which yoga intervention exerts its cardiac and vascular protective effects. However, further studies are required to confirm this hypothesis.

Improved circulating inflammatory markers after yoga practice have been observed in clinical patients with heart failure [15, 16], breast cancer [17, 18], chronic inflammatory diseases, and overweight/obese subjects [19]. In our present study, although yoga practice had no effect on circulating IL-8, TNF-α, and MCP-1 levels in healthy subjects, *via* whole blood culture *ex vivo*, reduction in IL-6, TNF-α, and IL-1β secretion has been observed after yoga training. The whole blood culture method is based on an optimal dilution of the blood cells in medium and no unphysiological cell separation is involved; thus it represents a physiologically much more relevant environment for the cells. Our findings could suggest that yoga practice may reduce the inflammatory status at the whole blood culture level. It is possible that a longer-term yoga practice than the present study design is required to reduce circulating proinflammatory cytokines in healthy subjects. Furthermore, yoga group also demonstrated reduced IL-6, TNF-α, and IL-1β secretion following TLR2 agonist stimulation but not TLR4; this was also associated with reduced TLR2 protein expression in PBMCs after yoga intervention. Collectively, it is suggested that yoga practice could result in blunted TLR2 response. We are yet to determine whether a yoga-induced blunting of TLR2 response represents a positive change for the health status in the long run. Considering that chronic inflammation is one of the key mechanisms involved in the pathogenesis of MetS [14], in the

long term, a decrease in TLR2 response may exert a beneficial effect because it decreases the inflammatory capacity of inflammatory cells, consequently suppressing whole body chronic inflammation. Compared with the reported effects of endurance training on TLR4 expression in men [23], the lack of LPS induced IL-6 and IL-1β secretion, as well as no alteration in TLR4 protein expression after yoga practice in our present study, may be related to differences in the type of intervention performed (aerobic, resistance exercise versus yoga), the intensity of the intervention, and/or the population examined. Clearly, more mechanistic studies are required to explore how different types of yoga practice affect TLRs expression and/or function in immune cells not only in healthy subjects but also in subjects with MetS.

5. Limitations

Our study has several limitations. First, the population used in our study was small and young healthy female subjects, limiting its generalizability to other populations. Second, the technique for the measurement of EMPs has yet to be standardized, so comparisons across studies may not be appropriate. Third, although we have shown IL-6 and IL-1β levels from cultured whole blood, the circulating IL-1β and IL-6 levels were below detection limits as measured via Luminex® technology. We acknowledge that it may be difficult to fully compare all of the cytokine markers measured due to differences in measurement technique and the physiological source of the biomarkers.

6. Conclusions

A total of 8 wk Hatha yoga practice in healthy Chinese female subjects could improve markers related to MetS, including reduced fasting circulating insulin, cholesterol and LDL-cholesterol levels, and circulating CD31+/CD42b− EMPs, as well as reduced TLR2 response from whole blood culture. As yoga seems to be a relatively safe intervention, it can be considered as an ancillary intervention in the primary MetS prevention for healthy population.

Competing Interests

The authors have declared that no competing interests exist.

Authors' Contributions

Zhongxiao Wan, Liqiang Qin, Neng Chen, and Xianghou Xia designed the study and wrote the final paper. Neng Chen, Xianghou Xia, Li Luo, Guiping Wang, Shufen Han, Ru Zhang, and Zhongxiao Wan conducted research and performed the statistical tests. All authors reviewed and approved the paper.

Acknowledgments

The authors thank the participants for their dedication and participation in the present study. This study is supported by the Natural Science Foundation of China (Grant no. 814729758 to Zhongxiao Wan), (Grant no. 81273067 to Liqiang Qin), and the University Science Research Project of Jiangsu Province (Grant no. 14KJD330002).

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Hindawi Publishing Corporation
BioMed Research International
Volume 2016, Article ID 8130731, 11 pages
http://dx.doi.org/10.1155/2016/8130731

Research Article

Cognitive Performance during a 24-Hour Cold Exposure Survival Simulation

Michael J. Taber,[1,2] Geoffrey L. Hartley,[1] Gregory W. McGarr,[1] Dessi Zaharieva,[1] Fabien A. Basset,[3,4] Zach Hynes,[3] Francois Haman,[5,6] Bernard M. Pinet,[7] Michel B. DuCharme,[4,8] and Stephen S. Cheung[1,4]

[1]*Environmental Ergonomics Laboratory, Department of Kinesiology, Brock University, 500 Glenridge Avenue, St. Catharines, ON, Canada L2S 3A1*
[2]*Falck Safety Services Canada, 20 Orion Court, Dartmouth, NS, Canada B2Y 4W6*
[3]*Department of Human Kinetics and Recreation, Memorial University, St. John's, NL, Canada A1C 5S7*
[4]*Marine and Arctic Survival Scientific and Engineering Research Team (MASSERT), Canada*
[5]*School of Human Kinetics, University of Ottawa, Ottawa, ON, Canada K1N 6N5*
[6]*Nutrition and Metabolism Research Unit, Montfort Hospital Research Institute, Ottawa, ON, Canada*
[7]*Faculty of Health Sciences, University of Ottawa, Ottawa, ON, Canada K1N 6N5*
[8]*Defence R&D Canada, Quebec City, QC, Canada G3J 1X5*

Correspondence should be addressed to Michael J. Taber; mt@ca.falcksafety.com

Received 24 February 2016; Revised 20 May 2016; Accepted 24 May 2016

Academic Editor: Edward J. Ryan

Survivor of a ship ground in polar regions may have to wait more than five days before being rescued. Therefore, the purpose of this study was to explore cognitive performance during prolonged cold exposure. Core temperature (T_c) and cognitive test battery (CTB) performance data were collected from eight participants during 24 hours of cold exposure (7.5°C ambient air temperature). Participants (recruited from those who have regular occupational exposure to cold) were instructed that they could freely engage in minimal exercise that was perceived to maintaining a tolerable level of thermal comfort. Despite the active engagement, test conditions were sufficient to significantly decrease T_c after exposure and to eliminate the typical 0.5–1.0°C circadian rise and drop in core temperature throughout a 24 h cycle. Results showed minimal changes in CTB performance regardless of exposure time. Based on the results, it is recommended that survivors who are waiting for rescue should be encouraged to engage in mild physical activity, which could have the benefit of maintaining metabolic heat production, improve motivation, and act as a distractor from cold discomfort. This recommendation should be taken into consideration during future research and when considering guidelines for mandatory survival equipment regarding cognitive performance.

1. Introduction

Extreme tourism (i.e., Alaskan and Antarctic cruises) is becoming more popular as larger sections of polar ice cap melt. As a result of expanding marine traffic in polar waters, there is an increase in the possibility of ships grounding on areas of previously inaccessible shoreline. Evidence of this possibility can be seen in the number of incidences reported by the National Transportation Safety Board (NTSB), the Transportation Safety Board of Canada (TSB), and various news agencies. Table 1 shows that since the Majestic Explorer rammed into a rocky shoal on the Alaskan shoreline in 1982, leaving one passenger dead and several others injured, there have been at least 20 such events in which passengers may be faced with the possibility of evacuation into harsh environmental conditions.

It has been reported that a mass rescue operation in support of an evacuation of a cruise vessel in Arctic waters would require approximately five days to complete [1]. Although international regulations mandate the amount of food, water,

Table 1: Cruise ship emergency events by region.

Name of vessel	Year	Region	Evacuation method
Majestic explorer	1982	Arctic	Inflatable liferafts
Nieuw Amsterdam	1994	Arctic	Refloated
Star princess	1995	Arctic	Evacuated to another ship
Spirit of 98	1999	Arctic	Inflatable liferafts
Wilderness explorer	1999	Arctic	Refloated
Clipper adventure	2002	Antarctica	Freed by Chilean icebreaker
Mona lisa	2003	Arctic	Evacuated to another ship
Le conte	2004	Arctic	Evacuated to another ship
Wilderness adventurer	2004	Arctic	Evacuated to another ship
Clipper odyssey	2004	Arctic	Coast Guard assistance
Lyubov orlova	2006	Antarctica	Transferred to another ship
Nordkapp	2007	Antarctica	Transferred to another ship
MV explorer	2007	Antarctica	Lifeboats
Empress of the north	2007	Arctic	Coast Guard assistance
Spirit of Alaska	2008	Arctic	Coast Guard assist/transferred to another ship
Spirit of glacier bay	2008	Arctic	Evacuated to Coast Guard vessel
Ushuaia	2008	Antarctica	Evacuated to Chilean navy vessel
Antarctic dream	2008	Antarctica	Free by a research vessel
Ocean nova	2009	Antarctica	Evacuated to Argentine Navy vessel
Clipper adventurer	2010	Arctic	Coast Guard assistance
Clelia II	2010	Antarctica	Assisted by NG Explorer
Polar star	2011	Antarctica	Evacuated to Argentine Navy vessel
Sea spirit	2013	Arctic	Zodiac capsizes during shore excursion
Silver explorer	2013	Antarctica	Damaged by 18' large wave

and equipment International Maritime Organization (IMO) (2002): guidelines for ships operating in Arctic ice-covered waters (I:\CIRC\MSC\1056-MEPC-Circ399), these supplies are only required to last for three days. Previous research, conducted over significantly shorter periods, has shown that cognitive performance is impaired by environmental thermal stress [2]. At present, it is not known whether the cognitive abilities required to perform vital survival tasks will be diminished during long-term cold exposure, thereby reducing the possibility of rescue and ultimately survival from a polar abandonment from ship or air. More specifically, it is not known whether there are measures that can be employed by the survivors to mitigate the possible deficits in performance.

Therefore, in an effort to address some of these unknown aspects of long-term survival in cool conditions, this paper presents cognitive performance findings from a 24-hour experimental cold exposure protocol in which the participants were able to actively and voluntarily control the amount of physical activity (active engagement) required to maintain a level of performance perceived to be sufficient to complete a series of cognitive tests. Based on previous research and duration of the exposure, it was expected that the cool conditions would impair some aspects of cognition (e.g., working memory and executive functioning) while having no effect or enhancing other aspects (e.g., reaction time for simple tasks).

2. Methods

2.1. Participants. The experimental protocol and instrumentation conformed to the standards set by the Declaration of Helsinki and was approved by the Research Ethics Board of Brock University (REB #09-230). Participants were medically cleared (cardiac stress test) by a primary care physician for any cardiovascular or neuromuscular symptoms and provided written informed consent prior to taking part in the study. An inclusion criterion was regular occupational or recreational experiences with cold exposure, and participants came from various professional backgrounds.

2.2. Experimental Design. The overriding design goal was to have participants thermally stressed to near the limits of voluntary tolerance, complete with mild hypothermia and shivering activity, for an entire 24 h without significant participant dropout (see [3], for more detail). The experimental design was developed to replicate some of the basic survival conditions that might be expected following vessel abandonment in the Arctic. For example, there was limited access to food, water, mental stimulation, and opportunities to sleep. To simulate the environmental conditions and emergency supplies that may be available in a lifeboat or life raft, no blankets or pillows were provided during the single continuous session of 24 hours of cold exposure. Participants were free to stand or sit in the chamber (described below)

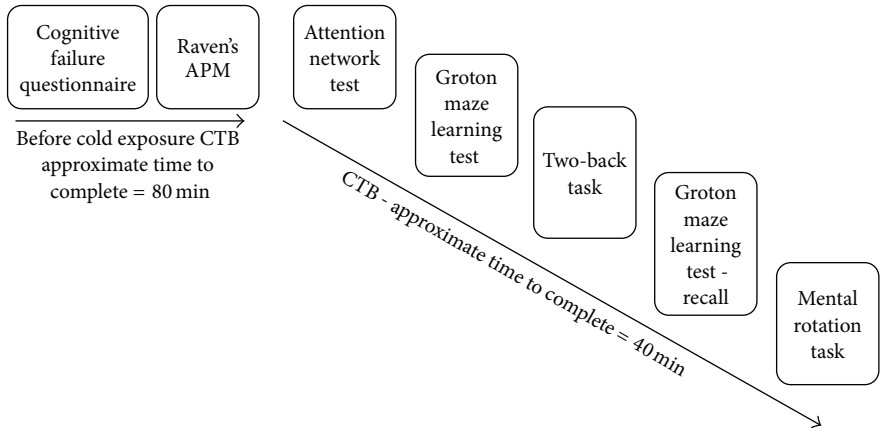

Figure 1: Full CTB administration protocol.

when they were not taking part in specific testing procedures. Prior to beginning and also following the 24-hour cold exposure, participants performed 60 min of moderate treadmill walking at 50% of their maximal aerobic capacity to simulate the level of physical exertion and activity that might be found during evacuation and rescue.

To test cognitive performance, a battery of tests that explored working and long-term memory, vigilance, absent-mindedness, general mental capabilities, executive functioning, information processing ability, and spatial ability in visual working memory was presented to participants at six different time points throughout the experimental protocol (baseline, 6, 12, 18, and 24 hours of exposure, and postexposure). Upon arrival on the testing day, participants completed the cognitive test battery (CTB) as a baseline measure. After exiting the environmental chamber, participants completed a final CTB following the postexposure treadmill exercise bout. The CTB was designed to explore both simple and complex tasks in an effort to identify where, if any, deficits might occur. Thermal comfort and sensation [4] were recorded at each CTB time point.

2.3. Establishment of Ambient Air Temperature Protocol. Given the limited research associated with long-term exposure, ambient air temperature used for the experimental protocol was based on pilot testing. Two participants completed different components of the experimental protocol for a planned 6 hours, with one additional participant completing a full 24 hours of testing. Based on the level of experiences described by the pilot participants and previous short-term cold exposure studies [5], an ambient air temperature of 5°C was used for the pilot studies. Body core temperature data and ratings of thermal comfort were then used to identify the likelihood that participants would be able to endure/tolerate the entire 24-hour exposure. Based on the results of the pilot testing and comments from the two participants, the experimental test protocol ambient temperature was set at 7.5°C (see [3], for more detail).

2.4. Cognitive Testing. To reduce the likelihood of a learning effect influencing the results collected during the actual administration of the CTB, a familiarization session was conducted several days prior to the cold exposure. This familiarization session gave participants the opportunity to practice sections of the CTB and ask questions about the test procedures.

As no previous research exploring cognitive performance during prolonged cold exposure exists, specific cognitive tests were selected to explore the influence of exposure on complex information processing requirements that might be used during a survival situation. To establish a baseline measure of cognitive self-evaluation and general fluid intelligence (Gf), participants completed a cognitive failure questionnaire (CFQ) [6] and Raven's Advanced Progressive Matrices (APM) [7] ~2 hours before entering the environmental chamber. These two tests were not completed after the exposure, as it is believed that a learning effect may take place during initial administration [8]. For example, Bridger et al. [9] reported a test-retest reliability of 0.71 for CFQs completed at 12 and 36 months after the original administration of the test. Given the relatively short time period (~24 hours) between the two test periods, it was expected that the participants would remember how they had responded on the initial (pretest) CFQ and Raven's APM.

To establish a measure of progressive changes throughout the cold exposure, participants were scheduled to complete the attention network test (ANT) [10], Groton maze learning test (GMLT) [11], two-back tests (TBT), and mental rotation tasks (MRT) at predetermined intervals (6 h, 12 h, 18 h, and 24 h) beginning 60 min after initial cold exposure (Figure 1). These measures were also compared against pre/postexposure scores. The GMLT and TBT are part of the psychometric measures available from CogState [12] and have been reported to correlate well (r's = 0.49–0.83) with similar psychological measures as well as show minimal practice effects [13].

This combination of tests in this integrated CTB has not been used before for cold exposure research; however,

all of the measures have been examined for reliability and validity in previous cognitive performance studies [11, 14, 15]. The following description of each test outlines the areas of cognitive function believed to be important to decision making in a survival situation.

Cognitive Failure Questionnaire (CFQ). The CFQ is a 25-item questionnaire that represents self-reported cognitive performance. The CFQ is measured as a total score ranging from 0 to 100 and is related to four factors of absentmindedness (memory, distractibility, blunders, and names). In addition, the CFQ items explore aspects such as spatial orientation failures, memory lapses, and motor functioning. Items are scored on a 5-point Likert scale where 0 equals "never" and 5 equals "very often." With high CFQ scores (scored above ~45), it would be expected that participants may have considerable difficulties completing tasks that require vigilance (e.g., ANT, Groton maze, and two-back tasks) in a prolonged cold exposure environment. The CFQ has been reported to have Cronbach's alpha value of 0.91 and a test-retest reliability of 0.82 over a 2-month interval [11]. Depending on the sample, an average CFQ score may be between 19 and 45 [16].

Raven's Advanced Progressive Matrices (APM). The APM is a nonverbal assessment tool designed to measure general mental capabilities pertaining to observational skills, decision-making, problem identification, perception, and sense making [15]. A total of 36 design puzzles with one piece missing are arranged in an ascending order of difficulty and raw APM scores have been used as an indicator of fluid intelligence [17, 18]. Individuals are asked to select one of four possible choices that follow puzzle design pattern rules. For the purposes of obtaining a baseline measure of cognitive ability, the APM was completed without a time limit [18].

Attention Network Test (ANT). The ANT is a psychometric tool used to test the efficiency of three distinct components of the human attention network (i.e., alerting, orienting, and executive control). The test is a combination of a flanker task with arrows and a reaction time task (for a full description of the test administration methods see [10, 19]). The ANT was selected for use during the cold exposure testing as it has been shown that executive functioning is impaired when core temperatures are reduced [20]. Reliability tests for consecutive ANT performance have shown that a learning effect exists for the executive functioning, as individuals progressively get better at ignoring incongruent signals [14]. Therefore, it would be expected that if the cold exposure has no effect on cognitive functioning, reaction times should improve each time the test is administered. As the ANT was found to be too onerous during preliminary pilot testing, it was only administered during the pre-, 6-hour, 18-hour, and posttesting sessions. Ishigami et al. [21] suggest that "overall RT is itself correlated with age ($r = 0.38$), the net- work [*sic*] scores ($r = 0.17$ and 0.33 for the orienting and executive scores, resp.; ns for the alerting scores), and also with the process scores (ranging from $r = -0.17$ to -0.52; ns for processes of Divided attention and Verbal monitor- ing [*sic*])" (p. 825).

Groton Maze Learning Test (GMLT). The GMLT is a computer-based neuropsychological measure (Cogstate, New Haven, CT) of working memory functioning (measured by the maze efficiency index) and information processing ability or executive functioning (measured by the number of errors) [22]. The test consists of a 10×10 grid of square tiles (covering a hidden pathway – 28 moves including 11 turns) presented to individuals on a touch screen computer surface. When presented to a participant, the GMLT is randomly selected from a test bank of 20 different versions of the maze, with each one equivalent in difficulty. Completing one test does not prepare the individual for subsequent GMLT, therefore, avoiding a learning effect between tests [23]. For each CTB session, the GMLT is presented six times (five initial repeated trials and one delayed recall requiring approximately 10 to 15 min to complete, which is used at the end of each of the CTB sessions to test working memory). Trials were timed (ms) and began automatically when the first move is made on the learning trial.

Two-Back Task (TBT). Similar to the GMLT, the two-back task (TBT) is a computer-based measure of visual working memory and attention (CogState, New Haven, CT). The TBT presents a playing card, shown face up, in the middle of a screen. Individuals are asked to decide (select "yes" or "no") whether a presented card is identical to one shown two cards before. An interstimulus interval of 2 seconds is used between the presentations of 35 cards. CR selected either the "d" (no) or "k" (yes) button on a standard QWERTY computer. Errors on the TBT have been reported to have a significant correlation with errors on the GMLT.

Mental Rotation Task (MRT). The MRT consisted of a computer-based (available at http://bjornson.inhb.de/?p=55) test of spatial ability in which an individual is given a number of visual choices that represent a rotated version of a master image. The master image consisted of small squares arranged into a pattern presented in an upright position. Each possible choice contains the same number of small squares; however, they are arranged in slightly different patterns (with the exception of one correct choice). Difficulty in selecting the correct match to the master image is created by rotating each of the choices a specific number of degrees to the left or right (e.g., 37° or −120°). The MRT was selected to explore the capability to maintain an understanding of spatial orientation of objects, which was believed to be important during the final stages of search and rescue operations (e.g., direction of aircraft in relationship to signaling devices).

2.5. Data Analysis. All data were examined with IBM SPSS (version 20) software. Prior to performing the statistical analysis for hypothesis testing, the data were plotted to check for errors and outliers. Additional checks were performed to test for the assumptions of normality (Shapiro-Wilkes test) and homogeneity of variance (Levene's test). Data collected from each of the CTB were compared for each of the dependent variables in a within and between subject design. Specifically, responses for each test were compared across

Table 2: Participant demographic and physiological information.

Participant	Measure							
	Age	Height (cm)	Weight before (kg)	Weight after (kg)	% body fat	USG before	USG after	$\dot{V}O_{2max}$ (mL/min/kg)
1	47	178.5	103.6	103.42	23.57	1.014	1.019	30.6
2	22	180	89.96	88.9	12.6	1.003	1.018	57
3	44	175.5	92.48	90.82	13.03	1.006		53
4	25	186.5	114.7	113.5	17.5	1.003		42.2
5	35	181.8	132.78	130.5	20.90	1.013	1.015	40.8
6	30	179.5	87.02	86.18	12.54	1.005		55.5
7	22	178	88.3	86.62	9.92	1.005	1.002	55.8
8	35	175.5	81.84	80.02	13.37	1.005	1.019	60
Mean	32.5	179.41	98.83	97.50	15.43	1.006		49.36
(SD)	(9.55)	(3.58)	(17.26)	(17.12)	(4.74)	(0.004)		(10.29)

Table 3: Participant cold exposure experience.

Participant	Cold exposure experience			
	Years of experience	Coldest temperature (°C)	Duration of exposure (hours)	Last exposure
1	25	−56	11	Within last 6 months
2	5	−20	6	Within last 6 months
3	20	−15	8	Within last 6 months
4	24	−45	6	Within last 6 months
5	27	−30	5	Within last 12 months
6	15	−25	1	Within last 6 months
7	15	−30	12	Within last 6 months
8	Did not respond to questionnaire			
Mean	18.71	−31.57	7	
(SD)	7.67	14.34	3.74	

the different time points for each participant to identify possible changes. These responses were also combined for all participants to identify general trends in the data based on the amount of exposure time. Repeated measures analysis of variance (ANOVA) was used to explore changes in CTB score for each test. Post hoc analyses were carried out where appropriate and alpha levels were adjusted according to Bonferroni corrections.

3. Results

3.1. Initial Cognitive Assessment (CFQ and Raven's APM). The results from the CFQ and Raven APM were used as measures of standardized cognitive processing. Due to known learning effects, these tests were only performed at baseline and were not repeated within this study. The mean CFQ score was 38.36 (SD = 8.75) (within the normal range for North American population) and all recorded CFQ scores fell within the 95% confidence interval [24]. The untimed administration of the Raven APM scores ranged from 17 to 35 with a mean of 22.9 (SD = 6.2). The scores were found to be normally distributed and based on standardized norms for a North American population [18]; the scores range from the 39th to >99th percentile.

3.2. Responses to Exposure. Given the intense nature of the applied research setting, participant sample size varied throughout the test protocol based on voluntary dropout. Tables 2 and 3 provide participant demographic information as well as an overview of previous occupational or recreational exposure to cold environments. Although the exclusion criteria were designed to limit the dropout rate, these fluctuations in sample size reflect situation in which it might be expected that within a given population forced to evacuate a commercial airliner or cruise ship some individuals may not survive until rescue arrives.

Given that the participants were recruited for their past experience, Table 3 details the relevant cold exposure information. From the table it can be seen that on average the participants have more than 18 years of cold exposure experience in temperatures ranging from −15°C to −56°C.

Subject Rating of Difficulty. On a subjective rating of difficulty where zero represented not difficult at all and 10 represented extremely difficult/need to withdraw from the study, participants rated the experience as an 8. Seven of the eight (88%) individuals indicated that there was at least one point throughout the trial that they believed they would have to voluntarily withdraw from the testing. Two

of the participants voluntarily removed themselves from the experimental protocol—one at 6.5 h and the other at 13 h. CTB results include the scores of these two participants for the period in which they remained in the trial (i.e., during the baseline and first 12 hours). Another participant performed the entire 24 hours but required the use of the thermal blanket from 16 h onwards. Overall, this suggests that the conditions were sufficiently taxing physically and mentally even for this motivated and self-selected participant pool. Seven of eight participants reported minimal sleep (~1.5 h) over the 24 hours. None of the participants found the bedspace to be a safe haven or comfortable, and most declined to use the bedspace or prematurely removed themselves from it over the course of cold exposure.

Core Temperature (T_c). No participants were removed from the experiment due to core temperature reaching 35.0°C, though two participants used the thermal blanket at various points throughout the testing. For most participants, core temperature generally decreased ~0.6°C (SD = 0.3) within the first 12 h of exposure and then stabilized at that approximate level for the remainder of the 24-hour exposure. Fluctuations within a range of 0.5°C occurred during this latter "stable" period, but overall the participants were able to sufficiently thermoregulate through shivering and some active engagement of mild exercise. Overall, this drop in core temperature was found to be significant ($F_{(4,32)} = 6.99$, $p < 0.001$), with a ~0.4°C (SD = 0.4) drop in core temperature between the baseline (pretest) and all other time points (6, 12, 18, and 24 hours of exposure). A similar examination of both thermal comfort and sensation did not reveal any significant differences across the 24 hours of exposure. Importantly, the thermal exposure also eliminated the typical 0.5–1.0°C circadian rise and drop in core temperature throughout a 24-hour cycle [25], such that the true level of hypothermic strain exceeded the ~0.4°C absolute T_c decrease for much of the exposure.

ANT Results. The individual mean scores of the three networks: alerting, orientation, and conflict, were 32.9 ms (SD = 18.3), 53.0 ms (SD = 23.9), and 138.1 ms (SD = 31.4), respectively. The mean total reaction time for correct trials was 652.5 ms (SD = 64.9). The test was administered at four different time point: before cold exposure; 6 hours; 18 hours, and after cold exposure. A one-way ANOVA indicated that there were no significant differences between scores based on administration time for alerting, orientation, or conflict. No significant differences in total mean reaction time were found for any of the test blocks.

GMLT Results. As part of the computerized CogState portion of the CTB, the GMLT was completed a total of six times during the course of this study (before, 6 h, 12 h, 18 h, 24 h, and after). Given the test protocol, individual GMLT were presented to the participant seven times (initial test sequence, five consecutive presentations, and one recall presentation after completing the TBT). After the initial presentation, the final maze in the initial block (Test Code GMLT-5) error rate was compared with the recall maze (Test Code GMLT-R) using a Wilcoxon Signed Rank Test. No significant changes in performance were noted across the trials. However, when comparing the same two presentations of the Groton maze for the postexposure session, the results indicate that there was a significant difference between the number of errors committed ($Z = -2.37$, $p = 0.018$). Figure 2 shows that almost all of the participants committed more errors while completing the recall GMLT during the postexposure CTB session. No other significant findings were found.

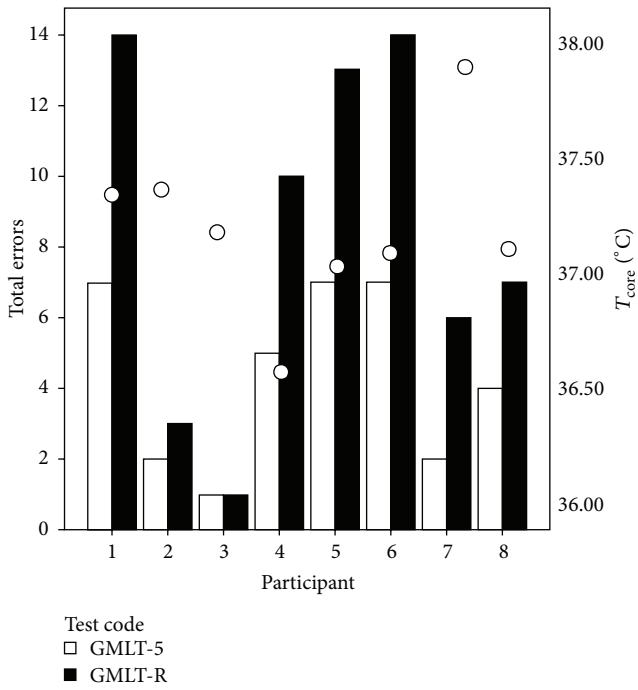

Figure 2: Total GMLT error based on GMLT-5 and GMLT-Recall during the postexposure session. The white circles represent the mean T_c of participants at the time of completing the recall GMLT.

TBT Results. The two-back task was administered during the same time points as the GMLT. A repeated measures ANOVA was conducted to explore the TBT speed, variability, accuracy, and number of errors. No significant findings were found regardless of trial administration time.

MRT Results. The mental rotation task (MRT) measured the accuracy, speed, errors, and time for the 10 images within each test block. Table 4 displays the mean reaction time (RT) and accuracy for each of the test blocks.

A Friedman Test revealed that there was a significant reduction in MRT accuracy ($n = 6$, $p = 0.04$). Post hoc analyses indicated that the significant difference occurred between first test after beginning the exposure (6 h) and after 18 h of exposure (Figure 3). Posttest results indicate that when completing the computer-based MRT, participants did not require significantly more time or commit more errors when compared to the pretest MRT. Results further indicated that pretest and posttest computer-based MRT correlate well with one another ($r(6) = 0.738$, $p = 0.037$). No other significant

Table 4: Mean (SD) reaction times and accuracy for the MRT based on test blocks.

Test score	Test block (hour)					
	Before	6	12	18	24	After
Reaction time (ms) (SD)	8.6 (2.5)	9.1 (3.6)	8.4 (2.1)	7.5 (1.0)	9.7 (2.8)	7.4 (1.8)
Accuracy (number of errors)	1.4 (0.3)	2.7 (1.6)	1.0 (0.6)	0.8 (0.8)	1.8 (1.2)	1.3 (0.2)

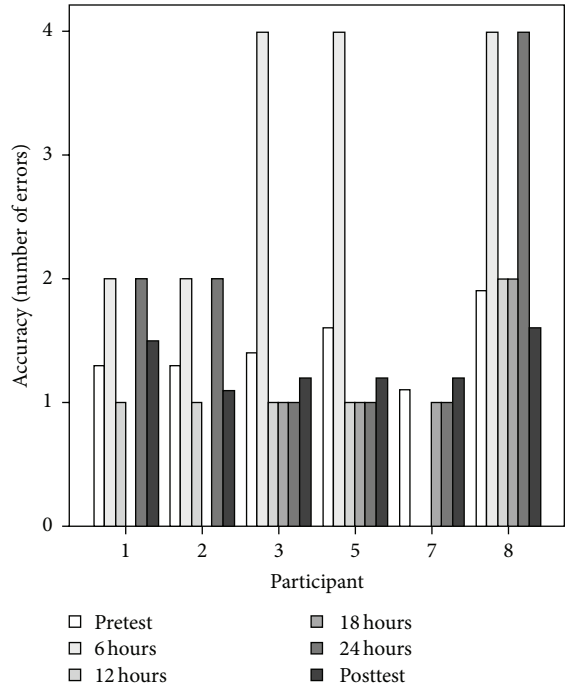

Figure 3: MRT errors committed by each participant across all trials.

findings were found for speed or accuracy regardless of the time at which the test was completed.

3.3. Combined Cognitive Effects. This section of the results addresses some of the combined effects of the experimental conditions on cognitive performance. Correlation analyses reveal that there were several significant relationships that existed within the CTB results; however, no effect of time was found for performance. Table 5 displays the correlation table for all of the tests as they relate to one another.

4. Discussion

This study aimed to simulate a prolonged survival scenario that might occur following a ship or plane incident in a cold environment. The primary goal was to elicit a sustained moderate thermal stress throughout 24 h, including a decrease in core temperature and elevation in metabolism through shivering. Additionally, we simulated many of the attendant situational factors, including isolation, boredom, food quality and availability, and sleep restriction. Rather than the restricted movement or voluntary physical activity in traditional hypothermia research, we permitted self-engaged physical activity within the confines of the testing chamber to replicate what might occur in a survival scenario. Overall, despite these challenging experimental conditions (confirmed by the participant difficulty ratings), cognitive performance (measured by the CTB) did not significantly alter throughout the course of the prolonged 24 h of cold exposure compared to baseline values taken before cold exposure. Together, this suggests that cognitive performance may be maintainable through sustained cold exposure, assuming that severe hypothermia can be avoided.

Despite previous cold exposure research showing a decrement in simple and choice serial reaction times, memory, sustained vigilance, and target tracking [26–28], others have shown that little or no changes in cognitive performance will occur over prolonged exposure if individuals are given the opportunity to self-regulate the amount of protective clothing worn or where exercise is used during the exposure sessions. For example, Slaven and Windle [29] showed that there were no significant decreases in cognitive performance in serial RT, Sternberg one-letter or seven-letter recall accuracy or speed. These findings were based on four days of consecutive testing in which the ambient air temperature was 15°C, 5.8°C, 4.4°C, and 4.4°C (resp.) and participants were able to select between three options of thermal protection. Similarly, Banderet et al. [30] found that over a five-day cold exposure session (ambient air temperatures ranged from −4°C at night to −25°C during the day) which included physical activity, significant differences in cognitive performance were only found for individuals who were hypohydrated at or below 2.5% of total body weight. In addition to the Slaven and Windle [29] and Banderet et al. [30] studies within an applied setting, Baddeley et al. [31] suggest that the lack of changes in cognitive performance found during exposure to 4.4°C water for approximately 60 minutes was due to highly motivated divers. Exposure times for all of these studies were considerably less than those carried out in this examination of cognitive performance.

Flouris et al. [28] show deterioration of vigilance and reaction time within the first 45 minutes of exposure to −20°C ambient air temperature, while a meta-analysis of cold exposure studies revealed that cognitive performance is decreased by an average of 14% in temperature at 10°C or less [32]. Meta-analyses [32, 33] have identified thermally induced reductions in cognitive performance that are most often observed when tasks are highly complex, require sustained vigilance, and place a considerable load on working memory. However, within these studies, the decrements to cognitive performance have been limited to specific domains such as working memory and vigilance [22], while effects have also been shown in long-term memory recall [26].

The disparity in research findings on the effects of cold-induced changes in cognitive performance has previously been explained by suggesting that the environmental stimuli (hot or cold ambient temperatures) act as a distractor [27, 34, 35] or as form of arousal [36, 37]. Based on the results from each of the cognitive tests, it appears that cognition was

Table 5: Correlation table for CTB administration.

	Correlations								
	CFQ	APM	Alerting	Orientation	Conflict	ANT RT	GMLT errors	MRT speed	MRT accuracy
CFQ score									
Pearson correlation	1								
Sig. (2-tailed)									
N	8								
APM score									
Pearson correlation	0.132	1							
Sig. (2-tailed)	0.755								
N	8	8							
ANT alerting									
Pearson correlation	−0.060	0.766*	1						
Sig. (2-tailed)	0.888	0.027							
N	8	8	29						
ANT orientation									
Pearson correlation	0.233	−0.195	0.249	1					
Sig. (2-tailed)	0.579	0.643	0.193						
N	8	8	29	29					
ANT conflict									
Pearson correlation	0.397	0.382	0.081	0.356	1				
Sig. (2-tailed)	0.330	0.350	0.674	0.058					
N	8	8	29	29	29				
ANT reaction time									
Pearson correlation	0.131	0.072	−0.036	0.587**	0.750**	1			
Sig. (2-tailed)	0.757	0.865	0.853	0.001	0.000				
N	8	8	29	29	29	29			
GMLT total errors									
Pearson correlation	0.477	−0.180	0.434*	0.298	0.089	0.194	1		
Sig. (2-tailed)	0.279	0.700	0.021	0.124	0.653	0.323			
N	7	7	28	28	28	28	319		
MRT speed									
Pearson correlation	0.447	0.062	0.031	0.332	0.150	0.218	0.295	1	
Sig. (2-tailed)	0.267	0.884	0.877	0.091	0.454	0.274	0.064		
N	8	8	27	27	27	27	40	43	
MRT accuracy									
Pearson correlation	0.775*	−0.244	−0.126	0.160	−0.076	−0.132	0.138	0.451**	1
Sig. (2-tailed)	0.024	0.560	0.531	0.425	0.705	0.511	0.396	0.002	
N	8	8	27	27	27	27	40	43	43

*Correlation is significant at the 0.05 level (2-tailed).
**Correlation is significant at the 0.01 level (2-tailed).

not significantly affected when examining overall changes during the long-term exposure. Given the paucity of thermoregulatory research assessing cognitive performance in long-term (more than 6 hours) cold exposure, it is interesting to note that Pilcher et al. [32] and Pietrzak et al. [22] reported that high intensity/short-term exposure had a greater negative influence on performance than less intense/long-term exposure during testing. These findings have been supported by research suggesting that cognitive performance above ambient temperatures of approximately 11°C will have minimal or no changes, whereas ambient conditions below this temperature result in deleterious effects [33]. Finally, Færevik et al. [38] reported that minimal physical activity can be used to minimize a reduction in core temperature over long-term cold exposure (24 hours). Together, this suggests that there exists a zone of optimal cognitive performance from

maintaining thermoneutrality, similar to that suggested by Hanin [36] and the extended-U hypotheses proposed by Hancock and Warm [37]. Both models suggest that there is a specific zone in which individuals will perform at maximal levels; however, performance becomes degraded if individuals are expected to perform tasks outside this optimal zone. Thermal stressors that do not increase the level of arousal beyond the optimal zone should, therefore, not be expected to adversely affect the performance of skills that are well rehearsed or require minimal cognitive effort.

One explanation for the limited changes to cognitive performance in this study is believed to be the amount of mild exercise performed by the participants and it was noted that individuals generally stood throughout the entire experimental protocol, indicating that it was too cold to sit for any length of time. It was observed throughout the cold exposure trials that participants would engage in mild physical activity such as hopping in one spot, swinging arms around their torso in a hugging motion, or vigorously rubbing their limbs after every test session that required them to sit for any period of time. Færevik et al. [38] showed that minimal exercise in cold conditions affects core temperature, suggesting that "5-min periods of moderate cycling leg movements every 20 min reduced shivering intensity, improved heat balance, slowed core cooling, and had a positive effect on the subjective perception of thermal comfort and reduced cold sensation" (p. 1000). Specifically, in an effort to explore the effects of exercise on body core while in water, Færevik et al. [38] reported that, in −2°C ambient air, 2°C water with 30–40 cm waves, participants rate of core cooling was significantly less when they performed moderate (sustainable) cycling for 5 minutes every 20 minutes. In fact, it was reported that not only did the moderate exercise decrease the rate of core cooling, it also significantly increased heat production to the point that a 10% gain was observed [38].

A second explanation for the results may be related to the experimental design of the project and appear to support previous research suggesting that minimal or no differences in cognitive performance should be expected when the intensity of the stressor (the cold ambient air in this case) is low [3, 22, 32, 33]. These findings also appear to support the results reported by Slaven and Windle [29] in which they describe that choice RT tests and short-term memory were unaffected after seven days in a simulated submarine in distress at 4.4°C. Similarly, Giesbrecht et al. [39] showed no significant difference in the performance of simple tasks based on cold exposure. Enander [27] noted that there were no decrements in simple RT tasks when participants were exposed to 5°C ambient air temperature over a period of 55 to 90 minutes (see also [39]). Participants in this prolonged cold exposure study were given the opportunity to engage in any activity that would help them stay warm enough to endure the full 24-hour exposure protocol. Despite the initial drop in core temperature, this active engagement potentially provides a coping mechanism during the testing and possibly could equate to a survival advantage in abandonment. There did not appear one definitive change in cognitive performance over the course of the experimental session. For example, there was no difference in attention related results (ANT and TBT), while there was a minor (nonsignificant) shift (more errors) in the working memory after 12 hours of exposure, and MRT results showed that, at 6 and 24 hours, there was a tendency for the participants to require more time to complete the questions and committed more errors (also not found to be significant).

Finally, it could be argued that another explanation for the findings is related to the level of stimulation present through the experimental session. The results might suggest that the level of arousal associated with the cold ambient air, constant shivering, cognitive testing, limited sleep, and confined conditions fell within an optimal zone of functioning for the selected group of participants [36, 37]. The only exception to this argument of the conditions falling within the optimal zone was found for the MRT errors. It is possible that the significantly higher number of errors in the MRT (when compared to the performance at 18 hours of exposure) could suggest that the level of arousal in the initial part of the testing was sufficient to influence the performance. As previously mentioned, the group of participants was specifically selected for this study to ensure a high success rate of completion. It may be that the intensity of the experimental protocol was ideally suited to provide a tolerable level in which performance was not affected [40]. For example, the ANT results were consistent with values reported by Weaver et al. [41] who found an overall mean reaction time (RT) of 646.5 (SD = 128.4), an alerting mean score of 33.0 (SD = 46.4), orienting mean score of 42.4 (SD = 37.4), and conflict mean score of 163.5 (SD = 90.0).

The results indicated that there were significant correlations between a number of the CTB measures. Given the specific cognitive tests used in this study and the reported validity, it would be expected that there would be strong correlations between and within particular components of the CTB. It was, however, somewhat unexpected that no changes occurred in the latter portion of the prolonged exposure. Given the fact that no time related correlations (e.g., negative relationship) were found, it can be assumed that the participants were sufficiently stimulated to overcome the expected influence on fatigue.

4.1. Main Contributions. As the majority of previous cold exposure research is conducted over considerably shorter periods of time (e.g., <6 hours), the primary contributions from this study are related to the extension of cognitive performance data over a much longer time frame. Additionally, the novel experimental protocol, which allowed individuals to actively engage in mild exercise, situates the data in more realistic conditions. For example, unless injuries preclude movement and assuming normothermic core temperatures as seen in this study, it is unlikely that survivors of a vessel abandonment will passively sit in one position while they continue to cool to the point that they no longer have the capability to help themselves.

4.2. Limitations. With no other changes despite a slight decrease in core temperature, constant shivering throughout

the exposure period, lack of sleep, and minimal food, it could be argued that it would be difficult to explain which factor(s) allowed participants to remain at a nearly constant level of cognitive performance. The limited number of participants tested in this study and the changes, both positive and negative depending on the type of measure, may have been due to fatigue or exposure or a combination of several other factors. Additionally, the individual differences in the responses to the GMLT may have obscured the effects of the cold response. Significant changes may also have been mediated by increased arousal levels associated with the distractive nature of the cold exposure [26].

4.3. Conclusions. In summary, despite a realistic survival simulation involving 24 hours of prolonged cold exposure, moderate decreases in core temperature, and sustained shivering, cognitive performance was largely maintained. This suggests that, as long as significant hypothermia is prevented, survivors may be capable of maintaining a range of simple through complex cognitive tasks for at least the first 24 hours of abandonment. One potential contributor to this performance maintenance may be the allowance of mild, self-engaged physical activity, which could have the dual benefit of maintaining core temperature and also improving motivation and acting as a distractor from the cold discomfort.

Competing Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

Acknowledgments

The authors are indebted to the participants for their spirit and enthusiasm throughout the study. The project was funded by a grant from the National Search & Rescue Secretariat (SAR New Initiative Fund supported by Transport Canada) through a contract from Public Works & Government Services Canada (Solicitation no. 31234-094495/A) and a grant from the Natural Sciences & Engineering Research Council of Canada (no. 227912-07, S. S. Cheung). M. J. Taber was supported by a Ministry of Research and Innovation Post-Doctoral Fellowship, and S. S. Cheung was supported by a Canada Research Chair. C. van Nijen assisted with data analysis.

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Hindawi Publishing Corporation
BioMed Research International
Volume 2016, Article ID 5613767, 8 pages
http://dx.doi.org/10.1155/2016/5613767

Research Article

Effects of Short-Term Physical Activity Interventions on Simple and Choice Response Times

Kevin Norton,[1] Lynda Norton,[2] and Nicole Lewis[1]

[1]*Faculty of Health Sciences, University of South Australia, Adelaide, SA 5001, Australia*
[2]*Faculty of Medicine, Nursing and Health Sciences, Flinders University, Adelaide, SA 5042, Australia*

Correspondence should be addressed to Kevin Norton; k.norton@unisa.edu.au

Received 18 February 2016; Accepted 28 March 2016

Academic Editor: David Bellar

Objective. Response time (RT) is important for health and human performance and provides insight into cognitive processes. It deteriorates with age, is associated with chronic physical activity (PA), and improves with PA interventions. We investigated associations between the amount and type of PA undertaken and the rate of change in RT for low-active adults across the age range 18–63 yr. *Methods*. Insufficiently active adults were assigned to either a walking ($n = 263$) or higher-intensity ($n = 380$) exercise program conducted over 40 days. Active controls were also recruited ($n = 135$). Simple response time (SRT) and choice response time (CRT) were measured before and after the intervention and at 3-, 6-, and 12-month follow-up. *Results*. SRT and CRT slowed across the age range; however, habitually active participants at baseline had significantly faster CRT ($p < 0.05$). The interventions increased weekly PA with corresponding increases in physical fitness. These changes were mirrored in faster CRT across the study for both intervention groups ($p < 0.05$). No changes were found for SRT. *Conclusions*. Both PA interventions resulted in improvements in CRT among adults starting from a low activity base. These improvements were relatively rapid and occurred in both interventions despite large differences in exercise volume, type, and intensity. There were no effects on SRT in either intervention.

1. Introduction

Response time (RT) is an important phenomenon in health and human performance. It is the time taken to physically react to a stimulus. Quantitatively it is the combination of processing time, or the time taken from the onset of a stimulus to the initiation of a volitional response, plus movement time [1]. In its most basic form it is called simple response time (SRT) and involves a single response to a signal.

The processing time combines the premotor period for the stimulus to reach the brain and the processing interval where activities such as recognition, association, coordination, inhibition, and decision-planning stages take place [2, 3]. These cognitive activities are especially important when there are multiple choices from which a potential response is made such as in choice response time (CRT) tasks [4]. Collectively, these (and other) more complex operations are often referred to as executive-control processes or executive functions [5].

Response time is important for health because it is associated with balance [6], the rate of voluntary stepping and mobility [7], probability of falls [8], and mortality [9, 10]. Slow RT is also related to driving errors such as collisions and traffic light violations in simulated driving tests [11]. For athletes involved in high-performance sport rapid anticipation, decision-making and movement speeds are critical for success and have been shown to discriminate the very best athletes from others [12].

It has been known for over a century that RT provides an insight into cognitive function [13]. This is because the central processing component of RT can be easily demonstrated and manipulated by varying the type and nature of the stimulus and response required. For example, simple RT is shorter than a recognition RT, and CRT takes even longer because the subject must choose a specific response corresponding to the stimulus. Declines in cognitive performance are associated with aging, brain-injury, and other neurodegenerative pathologies [14]. A meta-analysis of

age and cognitive function has found that around 75% of the age-related variance in several cognitive variables was also common with RT [15]. Physical activity (PA), however, has been shown to be associated with improved cognitive function in cross-sectional [16, 17], experimental [18–20], and longitudinal studies [21, 22]. Improvements have also been found in balance and frequency of falls in physically active intervention subjects versus less active controls [23]. A meta-analysis of aerobic exercise interventions and neurocognitive performance found modest improvements in processing speed and executive function [4]. Despite these findings there are still many unanswered questions concerning the relationships between PA and cognitive function and larger trials with longer follow-up are required [2, 4, 24]. In particular, as populations in many parts of the world continue to age and PA patterns remain low [25] there is a need to better understand the interaction of the amount and type of activity for optimal brain function and the time course for changes in cognitive function to take place using exercise interventions.

The study aims were to investigate the (1) relationships between RT and age in large cross-sectional cohorts of insufficiently active and regularly active participants and (2) effects of two types of PA interventions on RT in previously insufficiently active adults.

2. Materials and Methods

The methods used in this study are reported in more detail in Norton et al. [26] and in a paper reporting a range of other health and fitness changes as part of a large PA intervention [27, 28]. A brief overview is provided here.

2.1. Subjects. Ethics approval for this study was obtained from the institution's Research Ethics Committee. Adults aged 18–63 years were recruited following email advertising sent throughout a university, a tertiary hospital, and several government departments. Potential subjects were invited to attend the Exercise Research Laboratory at the University to complete informed consent and a questionnaire on their PA patterns covering the previous week [27, 28]. Those undertaking insufficient levels of PA for health benefits (InSA; <150 min/wk) were invited as intervention participants while the active subjects (SA; ≥150 min/wk) could volunteer to act as controls if this activity pattern was reported to be consistent over the previous 12 months. There were 312 insufficiently active subjects who were randomly assigned to one of two types of interventions: (1) a limited contact (information-oriented) pedometer-based strategy (n = 157) or (2) an active (instructor led) group-based strategy (n = 155) [26]. There were additional 106 subjects who could not attend the structured group intervention classes (for various reasons) but were otherwise willing to participate in the pedometer arm of the study giving a total of 263 pedometer participants. Further 225 nonrandomised participants, primarily from a university population, were recruited and undertook the group-based intervention giving a total of 380 group participants. There were no differences in the gender or physical activity patterns between the randomised versus nonrandomised subjects either before or after intervention and the additional nonrandomised subjects are, therefore, included in the present study. All intervention subjects were tested before intervention for baseline measures of RT. The intervention phase ran for 40 days at which time a postintervention test was conducted. Subjects then undertook follow-up testing at three, six, and 12 months after intervention with the exception of the additional group subjects who were only involved in the intervention phase of the study. The active control group (n = 135) was tested at baseline and six- and 12-month follow-up.

2.2. Interventions. Subjects in the pedometer intervention were equipped with a pedometer (Yamax Digiwalker SW-700) and a diary. They were instructed on how and when to wear the pedometer and were asked to record their step count at the end of each day. In the first week of the intervention subjects were instructed to achieve at least 5,000 steps each day, gradually increasing to 10,000 steps each day by the last week of the intervention. Weekly emails were sent to all pedometer subjects outlining the step count goal for the week and tips to increase walking activity [26].

The group intervention combined a number of elements that have previously been shown to be important for long-term behavioural change. Subjects attended the university three times each week for group activities led by instructors. Subjects participated in activity of their own choice on alternate days. Heart rate monitors (Polar S610, Polar Electro Oy, Finland) were worn during all activity sessions and subjects completed a daily diary of physical activity including type, duration, intensity, and rating of perceived exertion (RPE). Researchers downloaded HR monitors weekly to record exercise duration, % HR_{max}, and estimated energy expenditure. Group sessions were designed to have participants expend approximately 800 kJ/session in the first week and increase by about 200 kJ/session in each subsequent week. Activity sessions lasted 60 min with a core of about 40 min between approximately 75 and 85% of estimated HR_{max}.

Physical activity patterns, response time, and cardiorespiratory fitness were assessed for all participants at each test session. Minutes of PA during the previous week were quantified using the Active Australia Survey [27, 28] and no weighting adjustments were made for vigorous PA. Cardiorespiratory fitness was measured using submaximal assessment protocols on a bicycle ergometer [29] to predict maximal aerobic capacity [26].

2.3. Measurement of RT. On the test day subjects were instructed on how to use the RT equipment and the research staff made demonstrations of both SRT and CRT testing protocols. Subjects were then given several practice trials on the RT equipment to ensure they were familiar with the procedures before recordings were made. The SRT test involved the following sequence: subjects used the index finger of their dominant hand while seated in front of a keypad and computer screen; an audible "beep" was provided by the computer to indicate each trial was about to begin; a random fore period of between 1 and 3 seconds was used followed by a light switch being illuminated; subjects

Table 1: Descriptive data for the subjects. Physical activity (PA) data are total median (mean ± SD) minutes per week using REMM analysis. There were two participants from the group intervention arm who had missing preintervention RT data.

	Group	Pedometer	All intervention	Controls
n	380	263	643	135
Age at enrolment; mean ± SD (yr)	33.6 (12)	40.0 (13)	36.7 (13)	39.1 (12)
Gender (% F)	73	76	74	71
Preintervention PA (min/wk)	60 (60 ± 41)	60 (65 ± 42)	60 (62 ± 41)	405 (477 ± 270)
Postintervention PA (min/wk)	510 (556 ± 296)	270 (365 ± 308)	420 (478 ± 315)	
3-month PA (min/wk)	265 (309 ± 236)	220 (286 ± 238)	240 (293 ± 237)	
6-month PA (min/wk)	240 (284 ± 272)	240 (301 ± 275)	240 (294 ± 273)	395 (505 ± 388)
12-month PA (min/wk)	180 (270 ± 224)	200 (248 ± 211)	195 (256 ± 216)	365 (468 ± 366)

pressed a key as quickly as possible; and response time was recorded electronically to the nearest millisecond. The CRT test involved the same apparatus. Subjects sat in front of a keypad with four buttons and a random fore period was used together with a random illumination of one of four lights. Subjects pressed a button corresponding to the illuminated light as quickly as possible. In both tests ten trials were performed, the shortest and longest times were discarded, and the mean of the remaining eight trials was used in further analyses.

2.4. Statistical Analysis. Linear regression was used to determine the association between age and RT. Differences in intercepts and slopes between regression lines were calculated using t-tests. A longitudinal mixed model with random effects (REMM) imputation method was used to assess changes in RT over time [30]. This addresses the problem of missing data by using a model that estimates the trend shown by the subject over the available data and augments this by the trend from the whole sample [31]. This method is an intention to treat process and all data points are included in the models. The three groups were initially analysed separately and then all intervention subjects were combined for a separate REMM analysis. A significant group x time interaction effect indicated a significant difference in RT among (between) the groups across the study period. Alpha was set at 0.05.

3. Results

Descriptive details of the subjects are provided in Table 1. The mean age of the group intervention subjects was less than the pedometer and control groups ($p < 0.0001$). There were significantly more females than males who volunteered for this study but this was consistent across both intervention and control arms. The increased total PA levels were dramatic for both intervention arms across the 40 days of activity. The control subjects were highly active and proved to be a stable reference group across the duration of the study.

As expected, PA patterns decreased over the follow-up period although significantly higher PA levels were found for both intervention arms at 12 months after intervention relative to the low preintervention levels. The levels of vigorous activity during the interventions (minutes per week) increased from medians (mean ± SD) of 0 (6 ± 14) to 40 (74 ± 105) and from 0 (7 ± 15) to 240 (265 ± 164) in the pedometer and group arms, respectively. The elevated PA patterns reported by the subjects were supported by superior measures of cardiorespiratory fitness. VO_{2max} (mean ± SD in mL/kg/min) increased across the 40-day intervention from 27.2 ± 6.9 to 28.6 ± 8.2 and from 27.7 ± 6.9 to 32.4 ± 7.8, in the pedometer ($n = 209$; $p = 0.0003$) and group ($n = 283$; $p < 0.0001$) intervention arms, respectively. Using either REMM or per protocol analysis showed the control subjects were a very stable reference group for both PA patterns and cardiorespiratory fitness (Figure 1). For example, per protocol results showed the controls who completed both follow-up tests ($n = 96$) had baseline values of 38.1 ± 11.2 mL/kg/min and these were unchanged across the study (38.8 ± 10.8 and 38.3 ± 11.5 at the six- and 12-month tests, resp.; $p = 0.53$).

3.1. Response Time. Figure 2 shows the relationships of RT versus age and between participants who were active versus insufficiently active prior to the intervention. Regression analysis of all preintervention RT measures (using all subjects, $n = 776$) showed both SRT ($y = 224.8 + 0.25 * x$; $p = 0.038$) and CRT ($y = 300.1 + 1.60 * x$; $p < 0.0001$) increase across the age span. SRT deteriorated at a rate of about 1.1% per decade while CRT slowed at about 5.3% per decade. Comparison of the SRT versus age regression lines between the intervention and control groups showed no differences for slope ($t = 0.17$; $p = 0.87$) nor intercept ($t = 0.81$; $p > 0.05$). There was no difference in the regression slopes for CRT ($t = 0.37$; $p = 0.71$); however, there was a significant difference between intercepts ($t = 1.79$; $p < 0.05$) where the controls had faster CRT that remained consistent across the age span.

REMM analysis indicated there was no difference in the patterns of change in SRT among the three groups across the 12-month study period. Figure 3(a) shows there was a similar learning effect for all groups. Similarly, REMM results for the combined intervention participants versus controls showed no differences in group x time for SRT ($p = 0.168$). Figure 3(b) shows CRT decreased significantly for both the group ($p = 0.013$) and pedometer ($p < 0.0001$) participants with no change for the control group ($p = 0.056$). There was no difference in regression slopes between the group and pedometer participants ($p = 0.923$). REMM results

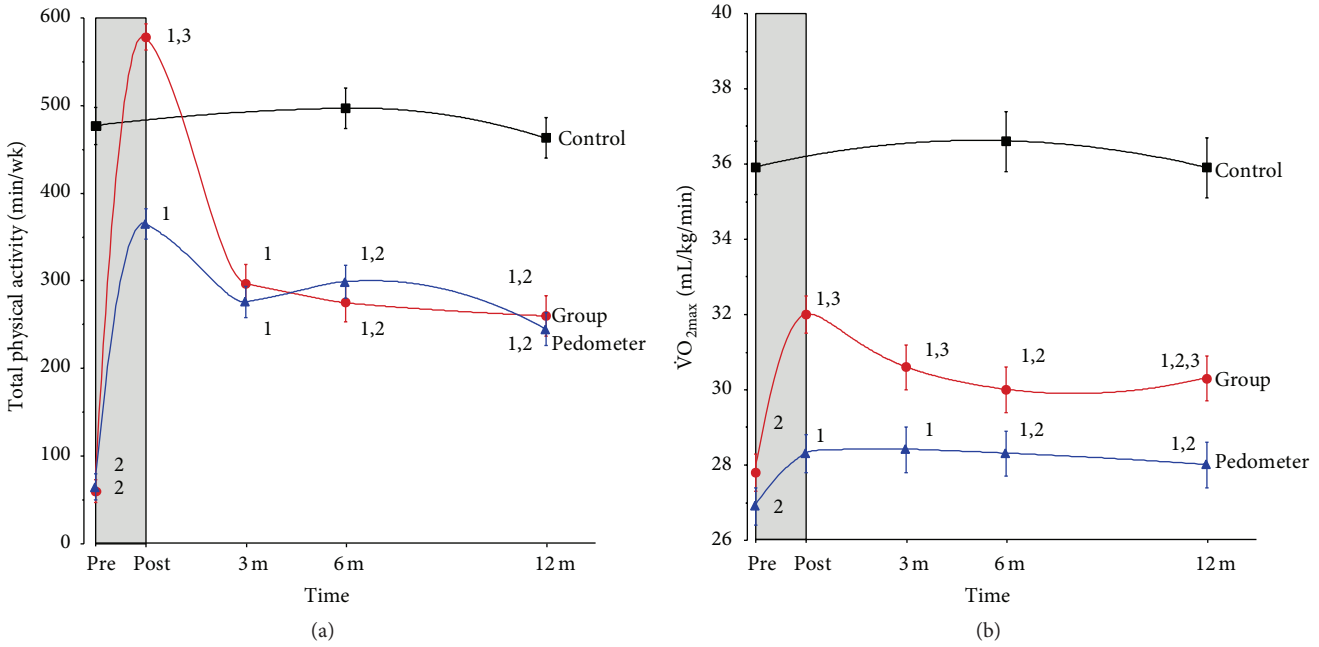

FIGURE 1: REMM results for PA and VO_{2max} across the study. The grey area represents the intervention phase. Values are mean ± SE. 1 = difference versus preintervention (within group); 2 = difference versus control; 3 = difference versus pedometer. Interpolation lines are computer-generated.

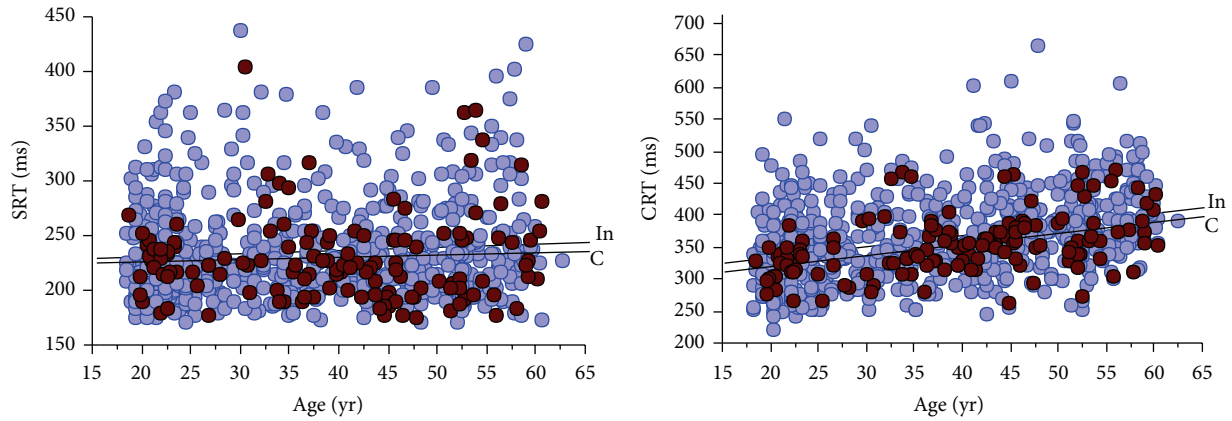

FIGURE 2: The change in RT with age. (a) shows there was no association in SRT with age for the control participants (controls (C) = darker circles; $n = 135$; $y = 221.4 + 0.20 * x$; $p = 0.478$) while there was a weak positive association for the intervention participants (intervention (In); $n = 641$; $y = 224.9 + 0.28 * x$; $p = 0.038$). (b) shows there were significant positive associations in CRT with age for both the control (controls (C) = darker circles; $n = 135$; $y = 285.4 + 1.74 * x$; $p < 0.0001$) and intervention participants (intervention (In); $n = 643$; $y = 301.7 + 1.61 * x$; $p < 0.0001$).

for the combined intervention participants versus controls showed a difference in group x time for CRT ($p = 0.028$). Overall, there was a medium effect size (0.55) in CRT for the intervention group from baseline to end (95% CI = 0.44–0.66), while the effect size for the control group (0.09) was not significant (−0.15–0.33).

4. Discussion

This is one of the largest studies reporting changes in RT across a range of ages following PA interventions [2, 4, 32]. Preintervention analysis using all participants showed both SRT and CRT slowed across the age range. However, participants who were habitually active at baseline had significantly faster CRT across the age range. No differences were found between rates of decline in either SRT or CRT between active and insufficiently active participants. The 40-day intensive PA interventions resulted in average increases in weekly activity patterns of about 6–9-fold for the pedometer and group-based programs, respectively. Correspondingly, the predicted VO_{2max} values also increased, averaging approximately 5 and 17% in these two intervention groups, respectively. These

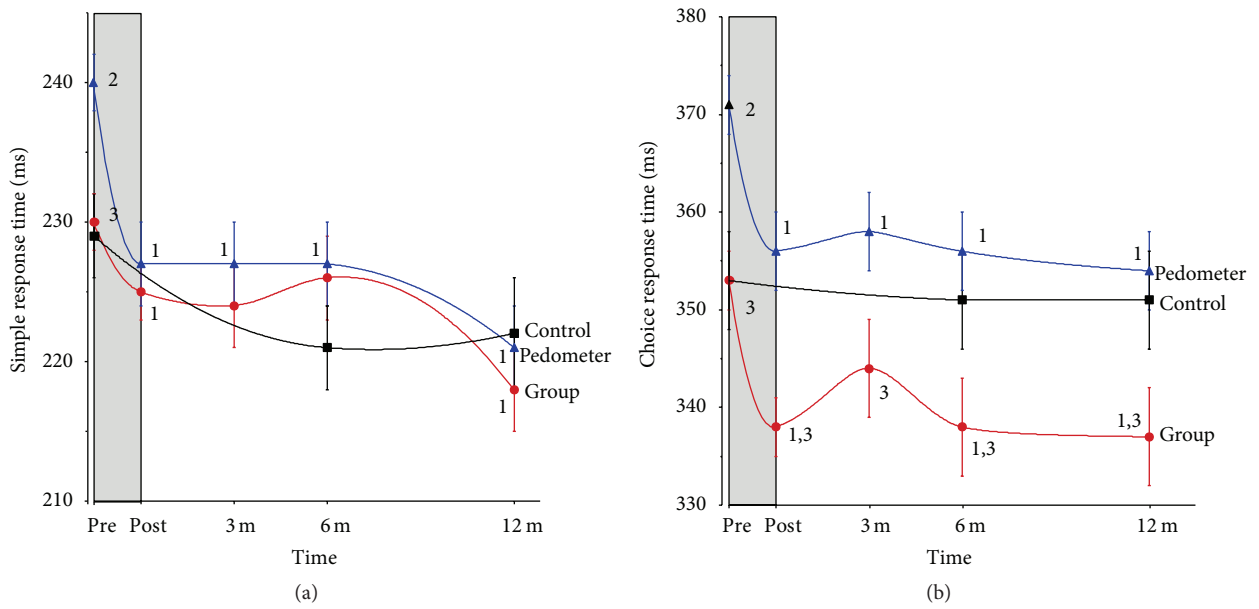

Figure 3: REMM results for the change in RT over the duration of the study. The grey area represents the intervention phase. Values are mean ± SE. 1 = difference versus preintervention (within group); 2 = difference versus control; 3 = difference versus pedometer. Interpolation lines are computer-generated.

results confirmed relatively rapid physiological responses to the increased physical loads. In turn, using REMM analysis and with adjustments for learning effects, the PA interventions improved CRT by an average of approximately 3.8 and 3.4% for the pedometer and group intervention participants, respectively. The PA interventions had no significant effect on SRT.

Previous research has found a relatively consistent pattern of slower RT versus age in both cross-sectional [13, 33, 34] and longitudinal studies [33, 35, 36], and almost all report increasing intra-individual RT variability with age [13]. The results are often curvilinear when the age range extends beyond about 50 years for SRT whereas increases have been shown to accelerate for CRT much earlier [13]. In the present study, however, nonlinear regression analysis was not significant for SRT versus age ($p = 0.064$ for a second-order polynomial). It was also not different from linear regression results for CRT where correlation coefficients were $r = 0.361$ and 0.360 with identical RMS residuals (0.053) for both linear and nonlinear regression analysis, respectively. Our relatively narrow age range of 18–63 yr may have accounted for the linear pattern of RT versus age. In a large cross-sectional study of over 7,000 participants, and using tests comparable to those in the present study, the rates of change in RT across the age range 20–60 years increased at about 2.2% for SRT and 5.1% per decade for a four-choice CRT test [13]. A study by Fozard and colleagues [33] of 1,265 participants also showed linear changes in SRT and CRT equivalent to increases of about 2.2% and 4.9% per decade, respectively, despite an age range from 17 to 96 years. The present study showed similar changes for SRT (1.1%) and CRT (5.3%) versus age. The significantly lower CRT for the physically active participants relative to those who were insufficiently active at preintervention across the age range is important and may represent effects of longer-term PA on RT. The results are also consistent with others who have reported physically active people performing better on cognitive tests including RT [17, 18, 20] and have less brain tissue loss with aging [2]. Given that CRT declines early in adulthood and that being active helps to attenuate these declines, it is important that people remain active throughout life for both cognitive and physical health. Physical activity has been shown to improve and maintain brain function, particularly attention, processing speed and memory [4, 37, 38], and motor performance [9, 39] and these are critical in our aging population for activities such as driving and independent living and as predictors of dementia [40], falls [41], and mortality [9]. Overall, the majority of observational studies have shown a positive relationship between PA or cardiovascular fitness and cognitive ability [16, 38, 42, 43] and human PA intervention studies have been associated with improvements in brain systems and performance [4, 32, 44] and increases in brain matter [45].

It is because of the enormous range of health benefits, including cognitive-related improvements, that health promotion campaigns focus on increasing population levels of PA [46, 47]. While the amount and type of PA required for cardiovascular health are relatively well established [48], less is known about the dose-response relationships for optimal brain health [32, 37, 38]. The present study investigated the effects of two types of PA interventions on RT in previously insufficiently active adults. The pedometer intervention participants increased PA across the 40-day intervention from a median of 65 to 365 min/wk while the group-led participants increased substantially more from 60 to 510 min/wk. Despite such impressive increases in PA and the corresponding increases in cardiorespiratory fitness (Figure 1), there were

no changes in SRT in either intervention arm. The lack of change in SRT has also been reported in many other studies [18, 23, 32, 49].

On the other hand, CRT showed significant improvements across the 40-day intervention phase of the study in a manner that essentially mirrored the changes in PA and fitness (Figures 1 and 3). Even though there were large differences in PA patterns between the intervention groups, the CRT changes were similar. The differences in PA volume during the intervention showed they were almost exclusively due to the higher levels of vigorous PA undertaken by the group participants (median of 240 versus 40 min/wk for the pedometer participants). This suggests that either the moderate intensity activity, lower levels of vigorous PA, or the combination was sufficient to enhance cognitive performance and discriminatory speed as measured in the CRT task for previously insufficiently inactive adults. It is not possible to further refine the specific thresholds of moderate or vigorous PA for these improvements. However, REMM analysis showed the improved CRT persisted even though the longer-term patterns of PA decreased. For example, at 12 months the median levels of total PA were 200 and 180 min/wk (median vigorous PA levels were 30 and 60 min/wk) for the pedometer and group intervention arms, respectively. The results, therefore, approximate the broad recommendations of at least 150 min/wk of PA to achieve health benefits [48]. A meta-analysis using 24 studies found an effect size for aerobic exercise and subsequent changes in attention and processing speed of 0.158 (CI = 0.055–0.260) and greater improvements for combined (aerobic and strength-based training) versus aerobic only interventions [4]. Our group intervention involved a range of activities including circuit and resistance training that would be categorised as a "combined" trial [26]. Since there were equally impressive improvements in CRT in both intervention arms, the combined activities made little difference in RT changes relative to the walking-based pedometer program.

5. Limitations

There was a significant age difference between the intervention groups. This may have impacted the potential to respond to a PA intervention. For example, the younger group intervention participants exhibited faster CRT before intervention and this might have limited their capacity to improve despite their impressive gains in PA. In other words, the additional vigorous and total PA for the group participants may have resulted in even greater changes if they were older and had slower CRT before intervention. The PA patterns are based on questionnaire responses and are therefore subject to social desirability bias. However, the measured cardiorespiratory fitness changes support the PA data as other reported health risk factor changes do [27, 28].

6. Conclusions

Our cross-sectional analysis showed age-related changes in both RT measures. CRT increased at almost five times the rate of SRT between 18 and 63 yr. The active control group had significantly faster CRT across the age range studied compared to the insufficiently active participants suggesting enhanced processing speed may result from chronic PA habits. This is supported in the present study by the findings that both types of PA interventions resulted in improvements in CRT among adults starting from a low activity base. These improvements were relatively rapid and occurred in both PA programs despite differences in exercise volume, type, and intensity. There were no effects on SRT in either intervention arm relative to controls.

Acronyms

CI: Confidence interval
CRT: Choice response time
HR: Heart rate
HR_{max}: Maximum heart rate
InSA: Insufficiently active
PA: Physical activity
REMM: Random effects mixed modelling
RPE: Rating of perceived exertion
RT: Response time
SRT: Simple response time
SA: Sufficiently active
SD: Standard deviation
SE: Standard error
VO_{2max}: Maximal oxygen uptake.

Competing Interests

The authors declare that they have no competing interests.

Acknowledgments

Funding for this study was provided by the Australian Research Council and the South Australian Department of Health.

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