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Immunoendocrine alterations following Marine Corps Martial Arts training are associated with changes in moral cognitive processes



Jacob A. Siedlik ^a, Jake A. Deckert ^a, Aaron W. Clopton ^a, Nicole Gigliotti ^b, Marcia A. Chan ^b, Stephen H. Benedict ^c, Trent J. Herda ^a, Philip M. Gallagher ^{a,*}, John P. Vardiman ^d

- ^a Department of Health, Sport, and Exercise Science, University of Kansas, Lawrence, KS, United States
- ^b Department of Pediatrics, Children's Mercy Hospitals & Clinics, Kansas City, MO, United States
- ^c Department of Molecular Biosciences, University of Kansas, Lawrence, KS, United States
- ^d Department of Human Nutrition, Kansas State University, Manhattan, KS, United States

HIGHLIGHTS

- Pilot study describes changes in moral cognition and associated endocrine responses.
- Catecholamine concentrations are correlated with measures of Marine Identity.
- Training disrupted leukocyte trafficking but did not activate an immune response.
- This has implications for moral decision-making capacity in high-stress occupations.

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ABSTRACT

Combined physical and psychological stress events have been associated with exacerbated endocrine responses and increased alterations in immune cell trafficking when compared to exercise stress alone. Military training programs are rigorous in nature and often purposefully delivered in environments combining high levels of both physical and mental stress. The objective of this study was to assess physiological and cognitive changes following U.S. Marine Corps Martial Arts training, Seven active-duty, male Marines were observed during a typical Marine Corps Martial Arts training session. Immune parameters, including immunomodulatory cytokines, and hormone concentrations were determined from blood samples obtained at baseline, immediately post training (IP) and at 15 min intervals post-training to 1 h (R15, R30, R45, R60). Assessments of cognitive moral functioning (moral judgment and intent) were recorded at intervals during recovery. There were significant fluctuations in immunoendocrine parameters. Peak endocrine measures were observed within the IP-R15 time interval. Distributions of circulating immune cells were significantly altered with neutrophils and all lymphocyte subsets elevated at IP. IFN- γ and IL-17a exhibited small, non-significant, parallel increases over the recovery period. Moral functioning was informed by different social identities during the recovery resulting in changes in moral decision-making. These data demonstrate that the Marine Corps Martial Arts Program induces significant alterations in lymphocyte and leukocyte distributions, but does not shift the balance of Th1/Th2 cytokines or induce a systemic inflammatory response. The program does, however, induce alterations in moral decision-making ability associated with the observed endocrine responses, even suggesting a potential interaction between one's social identities and endocrine responses upon moral decision-making.

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1. Introduction

Military physical training programs are generally rigorous in nature, and tend to involve periods of intense physical activity, psychological stress, sleep deprivation, and exposure to extreme environments [1]. The combined effects of these physical challenges and psychological

stressors on a trainee's immune system are complex in nature and at times deleterious as evidenced by wartime immunosuppression in active duty military personnel [2]. The focus of the Marine Corps Martial Arts Program (MCMAP) is the personal development of each Marine in a team framework using a standardized, trainable, and sustainable close combat fighting system that will both prepare them for, and acclimate them to, the rigors of training and deployments. The MCMAP is intentionally delivered in an environment characterized by periods of intense physical activity and psychological stress and is intended to

^{*} Corresponding author at: 1301 Sunnyside Ave, Lawrence, KS 66045, United States. E-mail address: philku@ku.edu (P.M. Gallagher).

develop the physical skills, decision-making abilities, and character necessary for the Modern Marine.

Scientists have recently begun examining the impact of combined physical and psychological stressors on the body – both in terms of physiological and cognitive responses [3-8]. This combination of stressors, referred to as a dual challenge model, reveals new questions related to how the body responds not only to acute bouts of combined mental and physical stressors, but also how the body relates to the chronic presence of distinct acute stress events. Recent studies comparing combined stressors with exercise alone have observed increases in catecholamines [4,6,7] and cortisol [3,8] in the dual-challenge groups compared to the exercise only controls. Stress hormones play a crucial role in mediating the immune response to various stressors with both adaptive and maladaptive results [9-13] and have been shown to impact cognitive function in a similar manner [14]. The immune system is heavily influenced by hormones, particularly cortisol (CORT), norepinephrine (NE) and epinephrine (EPI) [12,13], and exhibits a biphasic response to increases in hormone levels. Acute stress enhances immune function in the short term; however, the changeover from acute stress to chronic stress causes a selective suppression of T helper (Th) 1cellular immunity in favor of a shift toward Th2-mediated humoral immunity [12,13,15,16]. It is theorized that stress hormones, through their inhibition of cellular immunity, may place individuals at an increased risk for respiratory infections, or other infections where cellular immunity serves as the primary defensive mechanism [16].

Huang et al. [7] investigated the response of professional firefighters to combined physical and psychological stressors and found a greater increase of NE, EPI, and interleukin (IL)-2 in the dual challenge condition when compared to an exercise alone trial. IL-2 plays a central role in many components of the immune response including naïve T cell differentiation into Th1 and Th2 cells [17,18]. If stress-induced immune activation in response to dual challenge stressors does occur, it is important to quantify the immune response and investigate potential mechanisms of action. This is especially pertinent when considering the physical and psychological intensity of field training exercises and programs related to military service. Therefore the purpose of this study was to examine changes in the profile of catecholamines, cortisol, circulating immune cells and immunomodulatory cytokines following an acute bout of U.S. Marine Corps Martial Arts training. In addition to the physiological measures, the current study included a cognitive component to further explicate the aggregate impact of the MCMAP on cognitive processes. It is our hypothesis that the MCMAP will alter endocrine and immune cell circulatory profiles without triggering a systemic immune response. We do believe, however, that the physiological response will be associated with changes cognitive processes immediately following the training event. This is an exploratory, observational study designed to provide insight into the immunoendocrine and cognitive effects of standardized Marine Corps training.

2. Methods

2.1. Subjects

Seven male, active duty, newly enlisted U.S. Marines (age $=20\pm1$ yr; height $=179\pm8$ cm; mass $=75\pm6$ kg) volunteered for this investigation. All Marines had recently graduated Recruit Training and the School of Infantry and were reporting to the Marine Corps detachment based in Fort Leonard Wood, MO for formal training in their assigned Military Operational Skill (MOS). Subjects provided informed consent and completed a medical history questionnaire prior to participation. At the time of recruitment, subjects were instructed to maintain their normal physical activity and dietary patterns leading up to data collection. This implied that subjects would be in a non-fasted state at the time of data collection. All data collection was completed at the Marine Corps detachment based in Fort Leonard Wood, MO on 2014-10-03. This study was approved by the University Institutional Review Board

for Human Subjects and the U.S. Marine Corps Human Research Protection Program in Washington D.C.

2.2. Training session

The Marines arrived at the testing location at 0530 h for baseline assessment and began the training session at 0600 h. MCMAP training consists of approximately 30 min of Combative Conditioning (CC) involving a variety of exercises including but not limited to sprints, calisthenics and partner carries/drags. The CC component is followed by approximately 30 min of Combative Arts (CA) under the supervision of a MCMAP instructor. The CA portion involves skill instruction and practice at varying intensities. In addition to the physical stress of training, the Marine's performance is constantly being evaluated/corrected by the MCMAP instructor. Temperature and humidity during training were 20.5 °C and 100% respectively. The training session lasted 65 min.

2.3. Instrumentation

2.3.1. Heart rate analysis

Participants were fitted with a Zephyr BioHarness 3 (Zephyr Technology, Annapolis, MD, USA) for heart rate (HR) measures. Continuous HR measures were recorded at 1-second intervals during the training session. HR data were downloaded using the Zephyr BioHarness Log Downloader (version 1.0.29.0). Five training zones were defined as follows: zone 1 < 60% predicted maximum HR (HR $_{\rm max}$); zone 2, 60%–70% HR $_{\rm max}$; zone 3, 70%–80% HR $_{\rm max}$; zone 4, 80%–90%; and zone 5, >90% HR $_{\rm max}$. HR $_{\rm max}$ was estimated using the methods of Tanaka et al. [19].

2.3.2. Blood collections and analyses

Blood draws were performed by physician-approved allied health care provider using standard technique. Venous blood samples were obtained by venipuncture at baseline. Following the training session, an intravenous catheter (Braun, 18 g, 32 mm) was inserted into the antecubital vein, and a small bore extension set (Braun, 20 cm) was attached. Venous blood samples were collected immediately after training end (IP) and every 15 min for 1 h post-training (R15, R30, R45, and R60) in sodium EDTA, sodium heparin, serum separator tubes, or no anticoagulant Vacutainers as indicated. For each post-training blood collection, approximately 1 mL of blood (with saline from the extension set) was drawn into a discard tube prior to the sample draw.

NE, EPI, CORT, immunoglobulin (Ig)-G, IgM, complete blood count (CBC), neutrophil oxidative burst and lymphocyte subsets were determined from blood samples obtained immediately after training end (IP) and every 15 min for 1 h post-training (R15, R30, R45, and R60). Peripheral blood was collected in a serum separator tube (16 ml) for analyses of cortisol and immunoglobulins. Blood (6 ml) was collected in vials containing EDTA for analyses of complete blood counts (CBC) and lymphocyte subsets. Peripheral blood (16 ml into sodium heparin) was obtained for analyses of catecholamines and neutrophil oxidative burst function. Cortisol levels, IgG, IgM, and CBC were assessed commercially by immunoassay, immunoturbidimetric, and cytometry methods respectively (Quest Diagnostics Laboratories, Lenexa, KS). Catecholamines (EPI and NE) were assessed commercially by high performance liquid chromatography (HPLC) with electrochemical detection (Quest Diagnostics Laboratories, Chantilly, VA). Lymphocyte subsets including absolute and percent CD3, CD4, CD8, CD19, CD16/56 and total lymphocytes and neutrophil oxidative burst function were assessed commercially by flow cytometry (Quest Diagnostics Laboratories, St. Louis, MO; Quest Diagnostics Laboratories, San Juan Capistrano, CA).

Peripheral blood (8 ml) was collected into at tube containing no anticoagulant for analyses of granulocyte macrophage colony-stimulating factor (GM-CSF), interferon (IFN)- γ , IL-10, IL-13, IL-17a, IL-9, IL-1 β , IL-2, IL-4, IL-5, IL-6, IL-8, tumor necrosis factor (TNF)- α and creatine kinase (CK) at baseline, IP, R15 and R60. Serum for analyses was obtained

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