Physiology & Behavior 154 (2016) 76–82

Contents lists available at ScienceDirect

Physiology & Behavior

journal homepage: www.elsevier.com/locate/phb

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.

ARTICLE INFO

Article history:
Received 15 September 2015
Received in revised form 6 November 2015
Accepted 11 November 2015
Available online 12 November 2015

Keywords:
Marine Corps
Moral cognition
Lymphocyte
Cytokines

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.

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).

http://dx.doi.org/10.1016/j.physbeh.2015.11.011
0031-9384/

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) 1-cellular 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 ± 1 yr; height = 179 ± 8 cm; mass = 75 ± 6 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_{max}); zone 2, 60%–70% HR_{max}; zone 3, 70%–80% HR_{max}; zone 4, 80%–90%; and zone 5, >90% HR_{max}. HR_{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-1β, 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

after clotting by centrifugation at 2000 *g* for 10 min at 4 °C. Samples were stored at −80 °C until analysis. Peripheral cytokine concentrations were determined using a human cytokine magnetic bead panel kit (HCYTOMAG-60K-13) (Millipore, Billerica, MA, USA). The samples were detected and analyzed using a Luminex 200 System (Luminex Corp, Austin, TX). Creatine kinase (CK) enzyme concentrations were computed using a CK reagent kit (Pointe Scientific, Inc. Canton, MI). The samples were measured using a Synergy microplate reader (BioTek, Winooski, VT) at 340 nm at 1, 2 and 3 min. The average within sample coefficient of variance (CV) for all CK time points was 2.9% (CV range 0.7–5.8%). All ELISA procedures were performed according to the manufacturer's instruction.

2.3.3. Cognitive measures

All cognitive measures were evaluated using previously validated instruments [20–22]. Assessments of State-anxiety were reported through the Spielberger State-Anxiety Inventory (SAI) for Adults. Moral Identity was obtained through the five-item Internalization subscale of the Moral Identity Instrument [20]. Past research has indicated that it is this Internalization subscale – defined as the extent to which a person's moral identity is centered upon oneself – which is the most robust predictor of morally relevant behavior [22]. A second and third set of survey items captured the subjects' Marine Identity (i.e. the extent to which one identifies as a "Marine") and National Identity (i.e. the extent to which one personally identifies with the USA).

To gauge an outcome variable in cognitive, moral functioning, The Moral Functioning Continuum (MFC) was adapted from the Continuum of Injurious Acts [21]. The MFC represents a hierarchy from less severe to more severe aggressive actions. Here, the subjects responded on a continuum (1–7) as to whether they consider acts in hypothetical situations as legitimate or appropriate. The first set of hypothetical questions was asked in the context of the extent to which the subjects will judge the act as legitimate or appropriate (i.e. Moral Judgment). The second set of similar questions was in the context of the extent to which the subjects would intend to carry out the act in each situation (i.e. Moral Intention).

2.4. Statistical analyses

Bayesian generalized linear models with Markov Chain Monte Carlo (MCMC) estimation were used for data analysis using the *MCMCglmm* package [23] in R version 3.0.1 [24]. Time was modeled as a fixed effect, while intercept and slope of time were modeled as random effects for each participant. Baseline values were entered as a covariate in all models. Blood data were found to be normally distributed and a non-informative, uniform prior was used for analyses. Reported parameter estimates include the posterior mean and the 95% credible intervals (CI). Parameter estimates were interpreted as statistically significant if the 95% credible intervals did not include zero and pMCMC values calculated in *MCMCglmm* were less than 0.05. Finally, at each time collection period, multiple linear regression analyses were employed to investigate the relationship of moral judgment and moral intention with moral identity, national identity, Marine Identity, and state anxiety.

3. Results

3.1. Heart rate response to training

The MCMAP program generated a substantial exercise stimulus as demonstrated by the summative time in heart rate zone data. Mean predicted HR_{max} for study participants was 194 ± 1 bpm. Average HR during training was 143 ± 10 bpm compared to an average HR of 85 ± 6 bpm prior to training start. Average HR for the CC component was 151 ± 7 bpm whereas the average HR for the CA training was 134 ± 12 bpm. Summative time in HR zones for the overall training period were: zone 1, 10.1 ± 9.3 min; zone 2, 16.6 ± 4.7 min; zone 3, 13.9 ± 5.2 min; zone 4, 12.7 ± 2.5 min; and zone 5, 8.4 ± 5 min.

3.2. Blood parameters

3.2.1. Plasma volume shifts over time

Blood parameters are typically reported as concentrations with some variation in values attributable to changes in plasma volume. Shifts in plasma volume have been seen in response to either mental or physical stress [25] and to control for these changes plasma volume shifts were determined using the Dill and Costill calculations [26]. Plasma volume changes (%) were found to be significantly different across time points (−4.02 ± 6.99, 11.01 ± 5.4, 6.04 ± 8.13, 1.73 ± 1.07, and −3.42 ± 3.37 respectively). Adjustments for volume shifts were made using the methods of Bacon et al. [25].

3.2.2. Endocrine responses to training

Stereotypical hormonal responses to training including substantial increases in circulating catecholamines immediately post training were observed in this study. To quantify endocrine responses to the MCMAP blood measures were mapped and trend lines visualized with regression equations calculated (Fig. 1a–c). Summary endocrine measures following training are shown in Table 1. Catecholamines increased significantly immediately post training with an

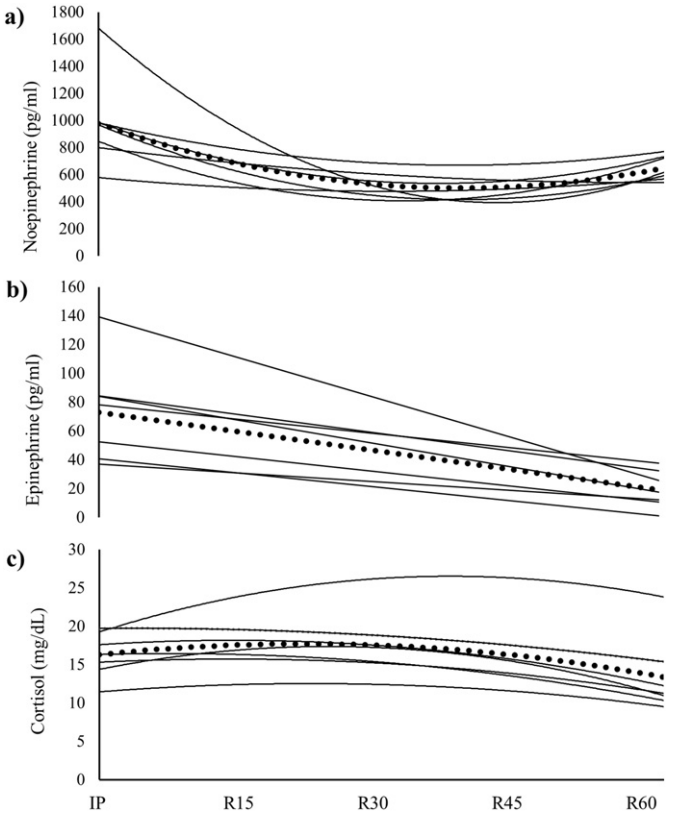

Fig. 1. a) Regression lines from the norepinephrine concentrations over time for each subject following MCMAP training. General regression equation: $\hat{y} = 1310.85\ pg \cdot ml^{-1} + 0.28(baseline) - 514.41\times + 71.98x^2$. 95% CI of slope $x = -772.41, -262.25$. 95% CI of slope $x^2 = 31.24, 113.35$. b) Regression lines from the epinephrine concentrations over time for each subject following MCMAP training. General regression equation: $\hat{y} = 85.07\ pg \cdot ml^{-1} + 0.08(baseline) - 13.49\times$. 95% CI of slope $= -19.92, -6.82$. c) Regression lines from the cortisol concentrations over time for each subject following MCMAP training. General regression equation: $\hat{y} = 8.08\ mg \cdot dl^{-1} + 0.31(baseline) + 3.3\times - 0.67x^2$. 95% CI of slope $x = -0.3, 6.58$. 95% CI of slope $x^2 = -1.24, -0.09$. Mean trendline = ···.

Table 1
Effect of Marine Corps Martial Arts Training on immune and endocrine parameters. Values are mean ± SD.

	Baseline	IP	R15	R30	R45	R60
Endocrine						
EPI (pg/ml)	26.1 ± 25.8	86.4 ± 53.3	49.3 ± 16.1	33.1 ± 18.6	35.9 ± 21.2	25.5 ± 19.2
NE (pg/ml)	397 ± 158	1009 ± 366.1	606 ± 101.4	565.9 ± 115.5	535.7 ± 107.1	634.1 ± 87
CORT (mg/dl)	17.8 ± 3	15.7 ± 3.2	19 ± 2.8	16.9 ± 5.5	15.6 ± 4.6	13.8 ± 4.9
Leukocyte and lymphocyte						
WBC (thousand/μl)	6.5 ± 1.8	9.2 ± 2.9	7.7 ± 2.2	7.4 ± 2.7	7.9 ± 2.4	8.6 ± 2.5
Neutrophils (cells/μl)	3709.0 ± 1172.3	5590.3 ± 1858.0	5339.7 ± 1680.9	5296.9 ± 2125.5	5726.2 ± 1804.6	6538.4 ± 2234.2
Eosinophils (cells/μl)	178.1 ± 178.5	187.0 ± 240.9	141.8 ± 177.4	148.7 ± 171.6	145.4 ± 193.3	109.3 ± 124.9
Basophils (cells/μl)	32.4 ± 18.3	42.4 ± 30.0	35.9 ± 13.6	28.1 ± 9.3	42.5 ± 39.0	35.2 ± 17.4
Monocytes (cells/μl)	488.9 ± 185.3	663.3 ± 348.9	491.1 ± 211.1	451.8 ± 209.6	462.7 ± 182.9	460.5 ± 149.0
CD3+ (cells/μl)	1390 ± 206.6	1613.4 ± 407.9	1080.5 ± 252.9	1030.4 ± 238.7	888.4 ± 324	982.6 ± 249.5
CD4+ (cells/μl)	796.3 ± 147	823.6 ± 163.7	639.7 ± 164.7	626.6 ± 147.9	535.3 ± 179.7	598.5 ± 152
CD8+ (cells/μl)	498.9 ± 105.5	752 ± 243.7	416 ± 121.1	397.2 ± 137.1	333.1 ± 129.8	363 ± 111.9
CD19+ (cells/μl)	310.6 ± 142.9	252.6 ± 84.7	212.6 ± 101.4	207.5 ± 74.5	169 ± 55.7	196.5 ± 76.5
NKC (cells/μl)	206.6 ± 117.2	644.6 ± 448.6	138.2 ± 55.5	107 ± 73.6	111.4 ± 79	129.4 ± 74.6
Immunoglobulins						
IgG (mg/dl)	1052.7 ± 195.2	1090.3 ± 203	1156.2 ± 188.9	1053 ± 212.7	1051.7 ± 216.6	1021.5 ± 201.9
IgM (mg/dl)	112.7 ± 64.7	121.5 ± 65.5	128.6 ± 73.3	115.5 ± 64.5	115.7 ± 64.5	109.9 ± 57.4
Oxidative burst						
Neutrophil oxidative burst (%)	94.7 ± 1.3	95 ± 1.9	96.1 ± 2.3	96.7 ± 1.5	93.2 ± 8.5	93.5 ± 8.2

average observed fold change of 3.3 in EPI concentrations compared to baseline values and a similar 2.5 fold increase in NE values at the same time point. Catecholamine concentrations decreased over the recovery period returning to at, or near, baseline levels by R60. These data indicate standard MCMAP training generates a strong sympathetic adrenal-medullary response. CORT concentrations remained relatively stable over the recovery period with only negligible fold changes observed. This may suggest that the training stress does not engage the hypothalamic–pituitary–adrenal axis (HPA). More likely, however, either the circadian variations are concealing the cortisol response [27] or the training response is diminished due to the Marine's non-fasted state [28].

3.2.3. Immune responses to training

Characteristic alterations in circulating lymphocytes and leukocytes were observed in response to the training stimulus with peak concentrations typically reached immediately post exercise and cell counts dropping below baseline during the recovery period. In order to identify patterns in immune cell trafficking trend lines were visualized and regression equations calculated for respective immune

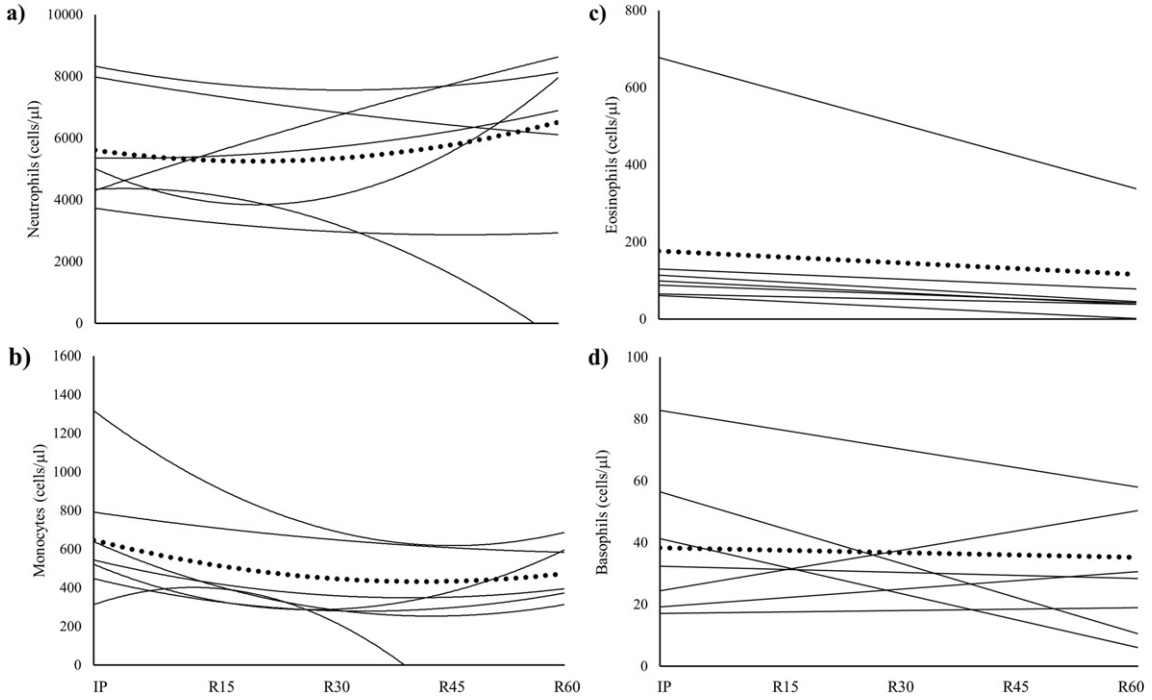

Fig. 2. a) Regression lines from the CD4+ cell counts over time for each subject following MCMAP training. General regression equation: $\hat{y} = 429.36\text{cells}\cdot\mu l^{-1} + 0.74(baseline) - 234.02\times + 29.73x^2$. 95% CI of slope $x = -407.34, -89.36$. 95% CI of slope $x^2 = 4.73, 58.04$. b) Regression lines from the CD19+ cell counts over time for each subject following MCMAP training. General regression equation: $\hat{y} = 161.27\ \text{cells}\cdot\mu l^{-1} + 0.47(baseline) - 61.45\times + 7.81x^2$. 95% CI of slope $x = -126.13, 2.15$. 95% CI of slope $x^2 = -2.63, 18.82$. c) Regression lines from the CD8+ cell counts over time for each subject following MCMAP training. General regression equation: $\hat{y} = 548.48\ \text{cells}\cdot\mu l^{-1} + 1.01(baseline) - 380.34\times + 49.03x^2$. 95% CI of slope $x = -563.81, -181.18$. 95% CI of slope $x^2 = 14.15, 77.67$. d) Regression lines from the natural killer (NK) cell counts over time for each subject following MCMAP training. General regression equation: $\hat{y} = 874.05\ \text{cells}\cdot\mu l^{-1} + 0.96(baseline) - 557.33\times + 74.86x^2$. 95% CI of slope $x = -877.44, -266.08$. 95% CI of slope $x^2 = 21.2, 124$. Mean trendline = ···.

measures. Total leukocyte and lymphocyte subpopulation responses to training are given in Table 1. For all measures the greatest increase in values was observed immediately post training. Baseline values were a significant predictor of measured responses for all parameters. Neutrophils (Fig. 2a), monocytes (Fig. 2b) and all lymphocyte subsets (Fig. 3a–d) exhibited a quadratic effect of time where values decreased from the IP peak until approximately R45 when cell counts began to rise. Eosinophils and basophils displayed approximately linear decreases over the recovery period (Fig. 2c & d).

Baseline and time were significant predictors for IgG and IgM responses following training. Both IgG ($\hat{y} = 99.32\ \text{mg}\cdot\text{dl}^{-1} + 0.99(baseline)) - 24.1\times$; 95% CI of slope = −42.6, −5.23) and IgM ($\hat{y} = 17.67\ \text{mg}\cdot\text{dl}^{-1} + 0.99(baseline) - 3.61\times$; 95% CI of slope = −6.18, −0.99) exhibited an approximately linear decrease over time during the recovery period.

Summary values for neutrophil oxidative bursts are given in Table 1. Oxidative burst capacity exhibited a small, non-significant decrease over the recovery period.

3.2.4. Cytokine and creatine kinase responses to training

None of the immunomodulatory cytokines included in this investigation showed significant increases in the recovery period following training suggesting that while MCMAP training generates significant alterations in circulating immune cells it does not activate an immune response. Of the 13 cytokines, eight (IL-10, IL-13, IL-9, IL-1β, IL-2, IL-4, IL-5, and IL-6) were undetectable in more than half of the serum samples and were excluded from the analyses. Summary data are presented in Table 2. For the cytokines analyzed (GM-CSF, IFN-γ, IL-17a, IL-8, TNF-α) baseline values were significant predictor variables for all but TNF-α. IL-17a and IFN-γ showed parallel increases over the recovery period but there were no statistically significant findings for slope over time (Fig. 4).

Creatine kinase is commonly used as a marker of skeletal muscle damage [29]. Analysis of CK measures over the recovery period yielded no significant results. While serum concentrations of CK do not usually peak until 1–4 days after a muscle damage protocol [30], our data suggest the MCMAP session did not cause substantial muscle damage.

Table 2
Serum cytokine and creatine kinase concentrations following MCMAP training. Values are mean ± SD.

	Baseline	IP	R15	R60
GM-CSF (pg/ml)	19.4 ± 25.9	16.3 ± 20.2	13.5 ± 20.5	14.7 ± 20.5
IFN-γ (pg/ml)	30.6 ± 57.3	31.8 ± 50.2	36.2 ± 60.6	39.4 ± 69.2
IL-17a (pg/ml)	11.3 ± 24.2	13.5 ± 23.1	15 ± 30.6	17.5 ± 34.6
IL-8 (pg/ml)	13 ± 13.2	13.3 ± 12.8	11.7 ± 13.9	13.1 ± 19.3
TNF-α (pg/ml)	7.8 ± 4	8.3 ± 4.2	6.7 ± 3.3	6.1 ± 3.6
CK (pg/ml)	210 ± 84.4	241.5 ± 65.6	273.3 ± 96.6	258 ± 87.9

3.3. Moral cognition during the recovery period

3.3.1. Associations between blood parameters and identity measures

Notably, at the 0 min and 30 min time periods, Marine Identity was inversely correlated with NE ($r_{0\ min} = -.79$, $p < .05$; $r_{30\ min} = -.85$, $p < .05$) and showed a similar inverse correlation with total catecholamines at the 60 min time point ($r = -76$, $p < .05$). Thus, the higher individuals identified as "Marines," the lower their NE and total catecholamines scores at the respective time points. Furthermore, at the 30 min mark CORT was negatively correlated with moral identity, where the greater the individual's CORT levels, the lower their responses to identifying as a "moral person," suggestive of poor decision-making in morally ambiguous circumstances. Finally, there was also a negative correlation between both moral judgment ($r = -.79$, $p < .05$) and moral intention ($r = -.80$, $p < .05$) with NE.

3.3.2. Predicting moral cognition over time

The use of social identities (i.e. Marine, National, and Moral) predicted one's moral judgment and intended behavior (i.e. intention) immediately following the training session while no connection existed 60 min post-training when levels approached baseline values. At

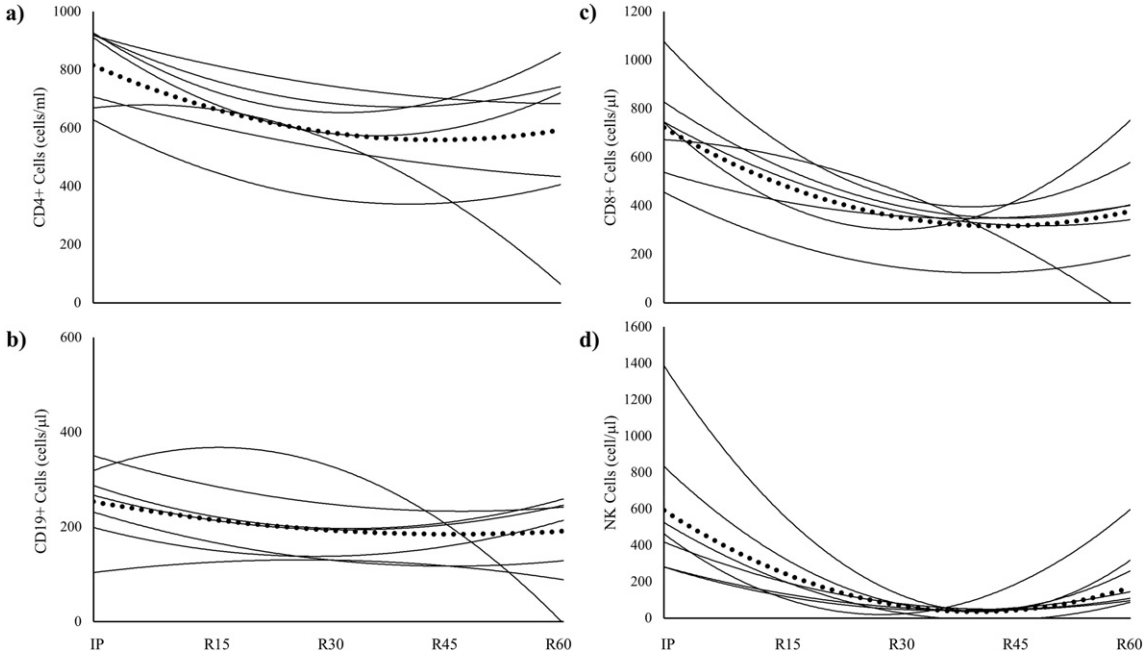

Fig. 3. a) Regression lines from the neutrophil counts over time for each subject following MCMAP training. General regression equation: $\hat{y} = 2211.73\ \text{cells}\cdot\mu\text{l}^{-1} + 1.1(baseline) - 851.75\times + 166.2x^2$. 95% CI of slope $x = -2283.75, 484.25$. 95% CI of slope $x^2 = -60.95, 409.31$. b) Regression lines from the basophil counts over time for each subject following MCMAP training. General regression equation: $\hat{y} = 21.61\ \text{cells}\cdot\mu\text{l}^{-1} + 0.58(baseline) - 1.71\times$. 95% CI of slope = −7.33, 3.96. c) Regression lines from the eosinophil counts over time for each subject following MCMAP training. General regression equation: $\hat{y} = 35.1\ \text{cells}\cdot\mu\text{l}^{-1} + 0.94(baseline) - 24.12\times$. 95% CI of slope = −38.09, −8.99. d) Regression lines from the monocyte cell counts over time for each subject following MCMAP training. General regression equation: $\hat{y} = 351.31\ \text{cells}\cdot\mu\text{l}^{-1} + 1.09(baseline) - 261.32\times + 33.86x^2$. 95% CI of slope $x = -418.56, -83.14$. 95% CI of slope $x^2 = 5.31, 60.7$. Mean trendline = ···.

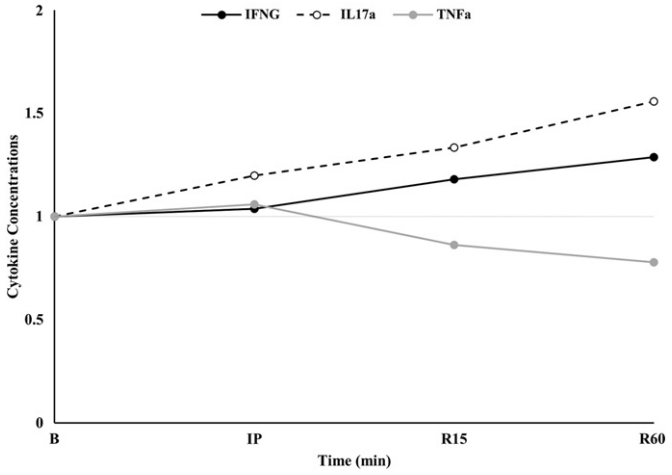

Fig. 4. Serum cytokine concentrations following MCMAP training. Values are normalized to baseline. IFN-γ general regression equation: $\hat{y} = -4.62\ \text{pg}\cdot\text{ml}^{-1} + 1.01(\textit{baseline}) + 4.73\times$. 95% CI of slope = −8.26, 15.24. IL-17a general regression equation: $\hat{y} = -4.85\ \text{pg}\cdot\text{ml}^{-1} + 1.17(\textit{baseline}) + 3.82\times$. 95% CI of slope = −6.88, 9.9. TNF-α general regression equation: $\hat{y} = 10.12\ \text{pg}\cdot\text{ml}^{-1} - 0.13(\textit{baseline}) - 1.23\times$. 95% CI of slope = −4.47, 3.24.

baseline, only one's national identity predicted their level of moral judgment ($\beta = -.60$, $p < .05$). However, at the IP mark, moral judgment was significantly predicted by one's Marine Identity ($\beta = .93$, $p < .01$), national identity ($\beta = -1.01$, $p < .01$), and current state anxiety ($\beta = -.37$, $p < .05$). At the same point, however, the extent to which each individual *intended* (i.e. moral intention) to act in an ethical/unethical manner was predicted by only Marine ($\beta = 1.04$, $p < .01$) and national identity ($\beta = -.99$, $p < .01$). The same identity variables remained significant at the 30 min mark for predicting both moral judgment (Marine Identity: $\beta = .91$, $p < .01$; National identity $\beta = -.72$, $p < .05$) and moral intention (Marine Identity: $\beta = .91$, $p < .01$; National identity $\beta = -.71$, $p < .05$). Notably, by one hour post-training, only the individual's state anxiety levels predicted either moral judgment ($\beta = -.51$, $p < .05$) or moral intention ($\beta = -.57$, $p < .05$).

4. Discussion

This exploratory analysis examined changes in immunoendocrine parameters and measures of moral cognition in active duty Marines participating in the Marine Corps Martial Arts Program. These data suggest a physiological – cognitive interface specifically related to moral functioning with social identities predicting moral judgment and intended behavior immediately following the training session. Moreover, significant correlations between NE, total catecholamine levels, and Marine Identity provide further evidence regarding the impact that this training had on the relationship between hormonal responses, cognition, and moral decision-making [14]. In fact, our results suggest a possible interaction might occur in the relationship between hormonal responses and one's cognitive process and its impact upon moral decision-making.

4.1. Peripheral immune response to training

No field studies have investigated immunological responses within the conceptual framework of a dual-challenge stress model. This study is a first step in quantifying the immunoendocrine response to dual-stress challenges in real-world training environments. Previous investigations of dual-challenge stress models in laboratory confines have found increased concentrations of IL-2 [6,7]. The current study was unable to reproduce those findings, with our data showing either undetectable or decreased values of IL-2 during the recovery period which is in line with a typical exercise response [31]. We did see a slight increase in TNF-α immediately post exercise followed by a decrease below baseline values over the remainder of the recovery period. Failure to find substantial increases in TNF-α is most likely a result of the moderate overall intensity of the training but may also be attributed to the short half-life in circulation (14–18 min) [32]. Future work may need to look at urinary samples for a better assessment of TNF-α concentrations in response to exercise.

As seen in Fig. 4, IL-17a and IFN-γ showed parallel increases over the recovery period. These paired increases were unexpected and, though small, are suggestive of activity outside of the typically studied Th-1/Th-2 paradigm. IL-17a is a pro-inflammatory cytokine derived primarily from Th-17 cells [33]; however, a subset of invariant natural killer T cells (iNKT) along with γδ T cells can secrete IL-17a when activated [34,35]. Variants of both these cell types have also been shown to secrete IFN-γ upon activation [35]. Catecholamines and cortisol suppress IFN-γ production [31] and it was unlikely that the increased concentrations observed were a result of an endocrine stimulus. That being said, Atanackovic et al. using an acute psychological stress model found increased concentrations of γδ T cells in peripheral circulation following the stress challenge [36]. That same study found no change in circulating iNKT cells [36]. Other work has shown γδ T cells to be stress responsive lymphocytes mobilized following either psychological or exercise stress in a dose-dependent manner correlated with cardiac activation and exercise intensity [37]. Examined collectively, there is reasonable evidence to warrant further research into pro-inflammatory cytokine production from either activated iNKT cell or influxes of γδ T cells following dual-stress challenges.

4.2. Moral function and endocrine associations

Cortisol was inversely correlated with Moral Identity at one point in recovery, with higher cortisol levels connected to lower levels of moral identity — or, presumably, lower levels of morality and morally-based decisions over time. That is, those with a resultant lower moral identity score would be more inclined to accept or perform behaviors that would be considered “less ethical” as defined in the Continuum of Injurious Acts [21]. Notably, Marine Identity was another cognitive variable that correlated with hormonal responses post-training. Here, the higher the individual identified as a “Marine,” the lower his NE and total catecholamine levels at the respective time points during the recovery period. Such a finding suggests that those Marines with high-Marine Identity might be more comfortable in a Marine-based setting and, therefore, would have relatively lower NE and total catecholamine responses as a result their comfort in the environment. Over time, and across varied situations, such a relationship might have a significant influence over one's decision-making processes particularly when confronted with morally-ambiguous situations. At the least, our mixed findings reinforce the notion that there is a need for further research within the dual-challenge stress model and to identify the structure of the variable constellation that impacts decision-making.

The MCMAP is designed to be physically and psychologically stressful, but is primarily a training environment that prepares Marines for the modern battlefield. That said, this training session should not be considered representative of stress resulting from active combat operations. It is interesting, however, that we observed shifts in cognitive processes in this controlled environment and we would expect changes to be exacerbated in response to higher intensity stressors. We anticipate future work will attempt to capture cognitive differences between individuals with abnormally high or low stress responses with the goal of understanding whether significant performance differences exist in these populations. It is our hope that this data, and future work in this area, will help military and first responder personnel train for that window during a stressful incident when decision-making processes may shift as a result of the physiological response. This is of special importance within the context of real-world operations where

individuals are repeatedly exposed to multi-factorial stressors in dynamic environments.

4.3. Limitations

Our goal to capture the immunoendocrine responses to actual Marine Corps training introduced limitations in the study design resultant from the operational tempo of the military environment. The research was conducted without placing dietary or activity restrictions on the participants prior to data collection. This allowed for us to make an accurate assessment of the physiological response to real-life training which we believe is the next evolution of dual-challenge stress research. Generalizability is hampered by the lack of control group and small sample size but, as stated earlier, this study was conducted as an observational, real-world analogue of laboratory dual-challenge stress models. We were fortunate in these analyses that the subjects we observed are all at the same point in their training pipeline (i.e. beginning formal MOS training) and as such have adhered to a fairly standard training protocol for the roughly 17 weeks prior to enrolling in this study as they completed Recruit Training and the School of Infantry. This allows for a relatively equal training status prior to their arrival and provides a basis for interpreting the data within the context of newly enlisted, male Marines entering formal school training. To our knowledge, this is the first study to quantify the immunoendocrine response to standardized Marine Corps Martial Arts Training and pair those data with measure of moral cognition.

5. Conclusion

In summary, we have shown that participation in the MCMAP induces elevations in circulating catecholamine concentrations and alterations in circulating leukocytes and lymphocytes without activation of an adaptive immune response. Additionally, Bayesian point estimates for cytokine data suggest increased concentrations of IL-17a and IFN-γ following training. Further investigation into cytokine expression outside of the Th-1/Th-2 paradigm will provide a better understanding of how the immune system responds to dual-stress challenges. Importantly we have also identified variations in cognitive function that suggest a link between physiological parameters and moral decision-making. Future research will need to focus on the direction of this interaction; namely, does comfort attained through an individual's identification with a given entity influence the endocrine response or is the endocrine fluctuation influencing identity considerations. A better understanding of the interplay between cognitive function and immunoendocrine interactions in dual-stress environments will improve not only our knowledge of disease susceptibility in military and first responder populations but also aid in the design of interventions aimed at improving the individual's decision-making ability in morally ambiguous scenarios common to modern military and law enforcement occupations.

Disclosures

Supported, in part, by a grant through the Office of Naval Research (ONR N000141410202) and a University of Kansas Doctoral Student Research Fund award.

References

[1] I.K. Brenner, Y.D. Severs, S.G. Rhind, R.J. Shephard, P.N. Shek, Immune function and incidence of infection during basic infantry training, Mil. Med. 165 (2000) 878–883.
[2] D. Gomez-Merino, M. Chennaoui, P. Burnat, C. Drogou, C.Y. Guezennec, Immune and hormonal changes following intense military training, Mil. Med. 168 (2003) 1034–1038.
[3] H.E. Webb, E.C. Fabianke-Kadue, R.R. Kraemer, G.H. Kamimori, V.D. Castracane, E.O. Acevedo, Stress reactivity to repeated low-level challenges: a pilot study, Appl. Psychophysiol. Biofeedback. 36 (2011) 243–250.
[4] H.E. Webb, R.S. Garten, D.R. McMinn, J.L. Beckman, G.H. Kamimori, E.O. Acevedo, Stress hormones and vascular function in firefighters during concurrent challenges, Biol. Psychol. 87 (2011) 152–160.
[5] H.E. Webb, M.L. Weldy, E.C. Fabianke-Kadue, G.R. Orndorff, G.H. Kamimori, E.O. Acevedo, Psychological stress during exercise: cardiorespiratory and hormonal responses, Eur. J. Appl. Physiol. 104 (2008) 973–981.
[6] C.J. Huang, H.E. Webb, R.K. Evans, K.A. McCleod, S.E. Tangsilsat, G.H. Kamimori, et al., Psychological stress during exercise: immunoendocrine and oxidative responses, Exp. Biol. Med. 235 (2010) 1498–1504.
[7] C.J. Huang, H.E. Webb, R.S. Garten, G.H. Kamimori, E.O. Acevedo, Psychological stress during exercise: lymphocyte subset redistribution in firefighters, Physiol. Behav. 101 (2010) 320–326.
[8] H.E. Webb, D.S. Rosalky, S.E. Tangsilsat, K.A. McLeod, E.O. Acevedo, B. Wax, Aerobic fitness affects cortisol responses to concurrent challenges, Med. Sci. Sports Exerc. 45 (2013) 379–386.
[9] E. Calcagni, I. Elenkov, Stress system activity, innate and T helper cytokines, and susceptibility to immune-related diseases, Ann. N. Y. Acad. Sci. 1069 (2006) 62–76.
[10] I.J. Elenkov, Neurohormonal-cytokine interactions: implications for inflammation, common human diseases and well-being, Neurochem. Int. 52 (2008) 40–51.
[11] M.E. Kemeny, M. Schedlowski, Understanding the interaction between psychosocial stress and immune-related diseases: a stepwise progression, Brain Behav. Immun. 21 (2007) 1009–1018.
[12] B.S. McEwen, The neurobiology of stress: from serendipity to clinical relevance, Brain Res. 886 (2000) 172–189.
[13] G.E. Miller, E. Chen, E.S. Zhou, If it goes up, must it come down? Chronic stress and the hypothalamic–pituitary–adrenocortical axis in humans, Psychol. Bull. 133 (2007) 25–45.
[14] F.F. Youssef, K. Dookeeram, V. Basdeo, E. Francis, M. Doman, D. Mamed, et al., Stress alters personal moral decision making, Psychoneuroendocrinology 37 (2012) 491–498.
[15] R.M. Sapolsky, L.M. Romero, A.U. Munck, How do glucocorticoids influence stress responses? Integrating permissive, suppressive, stimulatory, and preparative actions, Endocr. Rev. 21 (2000) 55–89.
[16] I.J. Elenkov, Glucocorticoids and the Th1/Th2 balance, Ann. N. Y. Acad. Sci. 1024 (2004) 138–146.
[17] W. Liao, J.X. Lin, W.J. Leonard, IL-2 family cytokines: new insights into the complex roles of IL-2 as a broad regulator of T helper cell differentiation, Curr. Opin. Immunol. 23 (2011) 598–604.
[18] W. Liao, J.X. Lin, W.J. Leonard, Interleukin-2 at the crossroads of effector responses, tolerance, and immunotherapy, Immunity 38 (2013) 13–25.
[19] H. Tanaka, K.D. Monahan, D.R. Seals, Age-predicted maximal heart rate revisited, J. Am. Coll. Cardiol. 37 (2001) 153–156.
[20] K. Aquino, A. Reed 2nd., The self-importance of moral identity, J. Pers. Soc. Psychol. 83 (2002) 1423–1440.
[21] B. Bredemeier, Moral reasoning and the perceived legitimacy of intentionally injurious acts, J. Sport Psychol. 7 (1985) 110–124.
[22] A. Reed 2nd, K.F. Aquino, Moral identity and the expanding circle of moral regard toward out-groups, J. Pers. Soc. Psychol. 84 (2003) 1270–1286.
[23] J.D. Hadfield, MCMC methods for multi-response generalized linear mixed models: the MCMCglmm R package, J. Stat. Softw. 33 (2010) 1–22.
[24] R.C. Team, R: A Language and Environment for Statistical Computing, R Foundation for Statistical Computing, Vienna, Austria, 2014.
[25] S.L. Bacon, C. Ring, G.Y. Lip, D. Carroll, Increases in lipids and immune cells in response to exercise and mental stress in patients with suspected coronary artery disease: effects of adjustment for shifts in plasma volume, Biol. Psychol. 65 (2004) 237–250.
[26] D.B. Dill, D.L. Costill, Calculation of percentage changes in volumes of blood, plasma, and red cells in dehydration, J. Appl. Physiol. 37 (1974) 247–248.
[27] J.A. Kanaley, J.Y. Weltman, K.S. Pieper, A. Weltman, M.L. Hartman, Cortisol and growth hormone responses to exercise at different times of day, J. Clin. Endocrinol. Metab. 86 (2001) 2881–2889.
[28] G. Brandenberger, M. Follenius, B. Hietter, Feedback from meal-related peaks determines diurnal changes in cortisol response to exercise, J. Clin. Endocrinol. metab. 54 (1982) 592–596.
[29] V. Mougios, Reference intervals for serum creatine kinase in athletes, Br. J. Sports Med. 41 (2007) 674–678.
[30] P.M. Clarkson, A.K. Kearns, P. Rouzier, R. Rubin, P.D. Thompson, Serum creatine kinase levels and renal function measures in exertional muscle damage, Med. Sci. Sports Exerc. 38 (2006) 623–627.
[31] K. Suzuki, S. Nakaji, M. Yamada, M. Totsuka, K. Sato, K. Sugawara, Systemic inflammatory response to exhaustive exercise. Cytokine kinetics, Exerc. Immunol. Rev. 8 (2002) 6–48.
[32] M.G. Davies, P.O. Hagen, Systemic inflammatory response syndrome, J. Surg. 84 (1997) 920–935.
[33] W. Jin, C. Dong, IL-17 cytokines in immunity and inflammation, Emerg Microbes Infec. 2 (2013).
[34] D.J. Cua, C.M. Tato, Innate IL-17-producing cells: the sentinels of the immune system, Nat. Rev. Immunol. 10 (2010) 479–489.
[35] J.A. Juno, Y. Keynan, K.R. Fowke, Invariant NKT cells: regulation and function during viral infection. PLoS Pathog. 8 (2012) e1002838.
[36] D. Atanackovic, U. Nowottne, E. Freier, C.S. Weber, S. Meyer, K. Bartels, et al., Acute psychological stress increases peripheral blood CD3 + CD56+ natural killer T cells in healthy men: possible implications for the development and treatment of allergic and autoimmune disorders, Stress 16 (2013) 421–428.
[37] L.H. Anane, K.M. Edwards, V.E. Burns, M.T. Drayson, N.E. Riddell, J.J. van Zanten, et al., Mobilization of gammadelta T lymphocytes in response to psychological stress, exercise, and beta-agonist infusion, Brain Behav. Immun. 23 (2009) 823–829.