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Cardio-respiratory and biochemical responses of military training event at Jungle environment

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ABSTRACT

Background: In military environment, soldiers regularly practice or undergo different types of extreme training activities. However, globally the literatures available on the physiological and biochemical demand of different extreme military training activities are very scanty and less reported. **Aims and Objective:** The present study was undertaken to quantify the cardio-respiratory and biochemical responses of military training event in jungle environment. **Materials and Methods:** Mathew's Mad Mile (MMM) activity is a type of specialized run of 1.5 mile in jungle environment. This training activity was conducted on rugged jungle terrain comprised of undulated uphill, downhill, muddy surface. Twenty-five SHAPE-1 healthy soldiers were volunteered into training event. Cardiorespiratory data was recorded continuously throughout the event and venous blood sample was drawn before and immediately after completion of the event. Statistical significance was considered at $p<0.05$. **Results:** There was no significant difference observed in heart rate and breathing rate while core body temperature was significantly ($p = 0.02$) higher in slow finisher as compared to fast finisher. In fast finisher, post exercise level of BDNF, BNP, SDH, cortisol and UCP1 increased significantly ($p<0.05$), whereas, BHB ($p<0.01$) decreased significantly in comparison to pre-exercise. In slow finishers, post exercise level of cortisol, KYNA and UCP1 increased significantly ($p<0.05$), whereas, BDNF, BNP and SDH decreased significantly ($p<0.05$), in comparison to pre-exercise. **Conclusion:** The outcome of this study indicated that the slow finishers were more susceptible to risk of injury due to higher exercise induced thermogenesis and mental stress in comparison to fast finisher.

Key words: Military training; Jungle terrain; performance; cardio-respiratory responses; Uncoupler protein-1; BDNF; Kynurenic acid

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INTRODUCTION

Jungle environment is hostile in terms of adverse climatic conditions (hot, humid and rainy climate), unpredictable terrain conditions (undulation, sliminess and submerged land) and presence of life-threatening flora-fauna. Beside these, unknowingness of whereabouts of enemies' attacking camouflaged positions into the dense jungle environment further put many-fold hurdles to the soldiers' performance. Under such circumstances soldiers' performance is regulated by interactions among the operational demands, environmental factors and individuals' physiological, neurohumoral responses. Much of the effectiveness of conventional jungle warfare depends on their familiarity

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with the terrain; lack of it may limit the ability of forces.[1] To meet the operational requirements, training is close to real time conditions through which soldiers can learn the techniques to counter these situations and the limits of existing performance of soldiers can be exceeded upto their maximum capability.[2] Thus, how well soldiers are able to tolerate operational and training demands and respond to multitude stressors over time has an impact on long term resilience and health.[3-5] Earlier studies have shown that highly resilient soldiers can cope better against negative effect of stress.[5,6] Therefore, the familiarity and adaptability of the soldiers can be increased by repeatedly exposing them in jungle environment through various rigorous training and conditioning methods. One such military training activity is Mathew's Mad Mile (MMM) and was considered in the present study. This activity is practiced in jungle environment with undulated uphill, downhill, muddy surface of rugged terrain. The general purpose of MMM is to train the soldiers, how to survive and perform better in the jungle environment. MMM exposes trainee to a multitude of absolute and relative stressors which may be faced in a survival or wartime situations.

Previous study revealed that increased muscular activity during exercise causes an increase in heat production in the body.[7] Only a small proportion of the heat produced in body is lost from skin affects evaporative loss of sweat due to additional stressor (environmental temperature and relative humidity).[7] The rise in core body temperature (CBT) is primarily depends upon the thermal gradients (difference between ambient temperature and skin temperature) and ability of individual to release heat into environment.[8] During fasting and vigorous exercise, G-Protein Coupled Receptor (GPCR) mediated activation of Protein Kinase-A (PKA) increases glycogenolysis in skeletal muscle and liver,[9] in addition to it, gluconeogenesis also stimulated through the transcriptional activation of numerous genes in the liver.[10] During gluconeogenesis, Beta Hydroxyl Butyrate (BHB) produces more ATP per mole of energy substrate than pyruvate, which could promote the expression of Brain Derived Neurotrophic Factor (BDNF) at glucose inadequate condition.[11] During glycogenolysis, succinate dehydrogenase (SDH) catalyzes the oxidation of succinate to fumarate in the Kreb's cycle. SDH plays an important role during high respiration rate and its activity is considered as a good indicator of the mitochondrial oxidative metabolic capacity during exercise.[12] The kynurenic acid (KYNA) increases energy utilization by activating GPCR-35, which stimulates lipid metabolism.[13] During lipid metabolism, Uncoupler protein-1 (UCP1) dissipates the most of the energy in the form of heat, which imparted role in increasing the exercise induced thermogenesis.[14] Previous study reported that the B-type Natriuretic Peptides (BNP) releases into the circulation to reduce the impact of exercise induced excessive thermogenesis by inducing vasodilation.[15] Bordbar et al. has mentioned in his study that BNP level was significantly increased after endurance exercise while significantly decreased after resistance exercise.[16] Cortisol is apparently required for coping with the higher energy demand as a result of the acute stress during exercise.[17] It was reported that low strength exercise did not change plasma level of cortisol whereas high-intensity exercise can elicit increment in blood plasma cortisol levels.[18] Plasma BDNF concentration accurately reflects brain BDNF levels compared to serum BDNF because serum BDNF is predominantly derived from clotted peripheral platelets.[19] BDNF can cross the blood-brain barrier in both directions, i.e. from the brain to the periphery and from the periphery to the brain. A number of studies have indicated that decreased levels of BDNF are associated with depression.[20,21] Thus, the concentration of BDNF in the plasma can be used to evaluate war fighters' stress induced depression due to high intense activity.

There is a need to understand how elevated level of stress impact performance of effectively trained individuals. Direct assessment of exercise induced metabolic demands during battle is not feasible. Understanding these demands during extreme military training activities may provide quantitative information on soldier's health status, which can be extrapolated to the responses elicit during combat/wartime situations. Thus, MMM offers an ideal avenue to understand the level of resilience of soldiers, is a unique opportunity for study. Hence, this study is unique in its approach, with an attempt to quantify the cardio-respiratory and biochemical responses of 'Fast finisher' and 'Slow finisher' undergoing MMM activity in jungle environment. It may be helpful to identify those individuals who are more susceptible to a prolonged stress prior to a significantly stressful event, could lead to a focused effort to improve performance.

MATERIALS AND METHODS

Participants

Twenty-five healthy soldiers were volunteered in this study. Participants wore full combat uniform along with the helmet.

Inclusion criteria

All the volunteers must be of SHAPE-1 standard and have at least two years of service period in the Indian army. Participants were well acclimatized to the study location in the past one month prior to commencement of study. They had no history of musculoskeletal or cardiovascular pathology. They were devoid of any ergogenic aid during entire period of study. All volunteers were briefed and written informed consent was obtained prior to participate in the study.

Exclusion criteria

Those who have not fulfil the criteria of SHAPE-1 standard, were not considered in this study.

Ethical approval

The study protocol conformed to the principles outlined by the Declaration of Helsinki protocol[22] and was also approved by the Institutional Ethical Committee (Ref no. IEC/DIPAS/D-1/2) on the use of human as study subjects.

Selection of event

Mathew's Mad Mile (MMM) activity is a type of specialized run of 1.5 mile in jungle environment. This training activity was conducted on rugged jungle terrain comprise of undulated uphill, downhill, muddy surface. Volunteers were thoroughly explained about the purpose of selection of activity and environment. The general purpose of MMM is to train the soldiers, how to survive and perform better in the jungle environment. MMM exposes trainee to a multitude of absolute and relative stressors which may be faced in a survival or war like situations.

Experimental design

Subjects reported to the training area in the morning. Baseline data were collected before commencing of the experiment/training event. Training event was conducted by a trained instructor from concerned training institute. As per the standard protocol of training, participants were supposed to run on tough jungle terrain and finished with specified time. On the basis of finishing time, their performance categorized as fast finisher (less than 16 minutes) and slow finisher (more than 20 minutes). Authors are unable to provide the details of training protocol due to confidentiality. The flowchart of the study adopted is given in Figure 1.

Preparation of subjects

Each participant was allotted a batch number before start of event. Participants were fitted with multi-individual physiological status monitoring system, (Bio-harness, PSM, Zephyr, USA) prior to participate in the event. A Bio-Harness belt was tied tightly at the fifth intercostal region of chest of the participants for the recording of physiological parameters.

Data collection and processing

The demographic data of all the participants were collected in front of training instructor in the training area before start of the main experiment. The environmental temperature and relative humidity (RH) of training area were 32-34°C, 75-90% respectively. The temperature and RH were recorded during training event using whirling psychrometer equipment (Dimple Thermometers, Delhi, India).

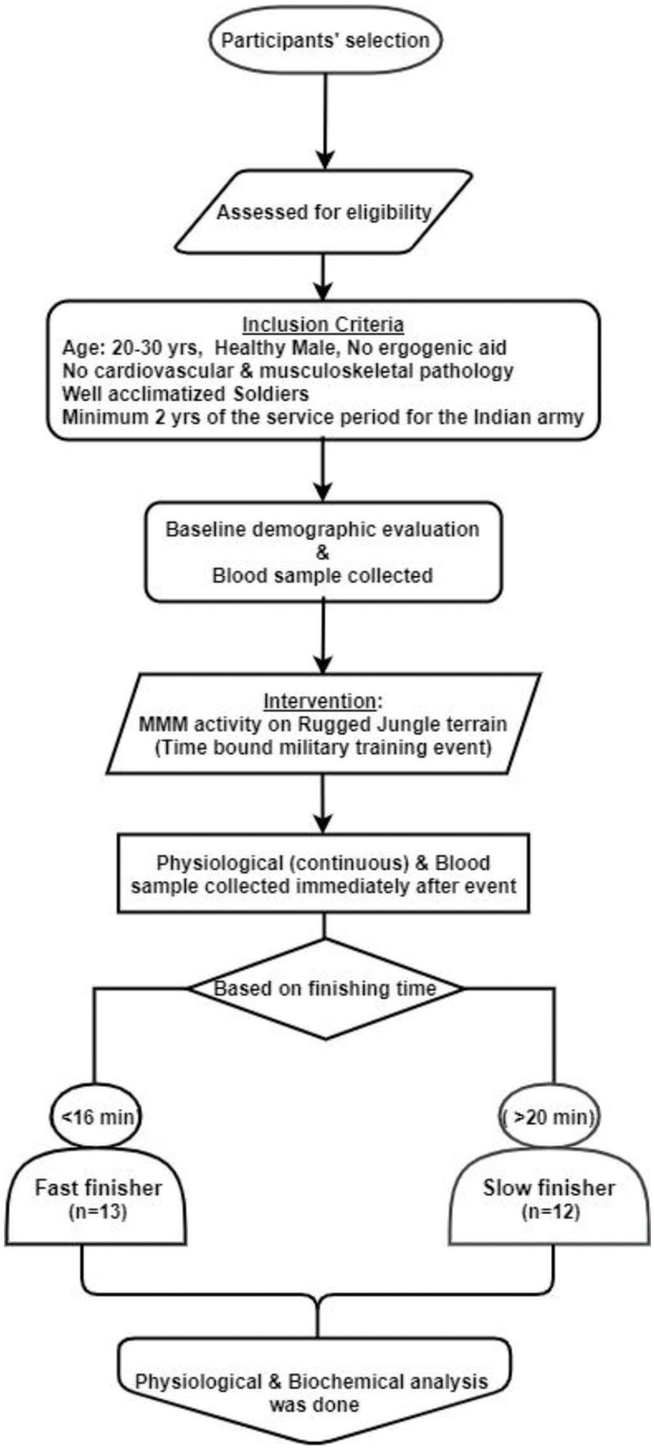

Figure 1: Flowchart of methodology adopted in the study.

Heart Rate (HR), Breathing Rate (BR), and Core Body Temperature (CBT) were recorded throughout the event. Continuous recording was obtained using 'Omnisense live' software and further exported into MS Excel using 'Omnisense analysis' software. Bit to bit data obtained was converted into per minute averaging format.

The venous blood was drawn by the allotted nursing assistant. Venous blood sample was drawn from each participant before and immediately after finishing the event. Approximately 5 mL blood was drawn from each subject in a heparinized 5 mL syringe and collected into EDTA coated tube. Centrifuged (BorosilPvt. Ltd.) the EDTA-tubes containing sample at 6000 rpm for 10 minutes and plasma was transferred into 2 mL Eppendorf tubes. Plasma containing tubes were placed in ice-cold condition and further stored at -80°C until analyzed. Plasma samples were subsequently analyzed for BDNF (ab212166), BHB (ab83390), BNP (E-EL-H0598), Cortisol (Cayman-500360), SDH (ab228560), KYNA (CED718Ge), UCP1 (SEF557Hu) using ELISA assay.

Statistics analysis

All variables were tested for normality with the Shapiro-Wilk and the homogeneity of variance using Brown-Forsythe test followed by parametric and non-parametric test accordingly. Pre and Post comparison within group with parametric variables was performed by Student's t-test and with non parametric variables Wilcoxon signed rank sum test was performed. Between the groups comparison with parametric variables was performed by Student's independent sample t-tests and with non parametric variables using Mann Whitney U test. All the statistical analysis was conducted on SPSS- V16 (Nikiski, AK 99635, USA). A level of significance was considered at $p < 0.05$.

RESULTS

There were no significant differences in Age, Height, Weight, BMI, RHR, SBP at baseline except DBP was significantly ($p<0.05$) higher in Slow finisher (Table 1). The time taken by Fast finisher (15.23±0.27 minutes) was significantly lesser ($p<0.0001$) in comparison to slow finisher (20.80±0.42 minutes). In case of cardio-respiratory responses there were no significant differences in HR, BR while CBT ($p=0.02$) was significantly higher in slow finisher (Table 2).

During comparative analysis between BE1vs AE1 of fast finisher found that the UCP1 ($p<0.001$) (Figure 2a), SDH ($p<0.001$) (Figure 2d), cortisol ($p=0.015$) (Figure 2e), BNP ($p=0.009$) (Figure 2f) and BDNF ($p=0.049$) (Figure 2g) level were significantly higher in AE1; while BHB ($p=0.007$) (Figure 2c) was significantly lower in AE1. There were no significant differences in the level of KYNA ($p=0.211$) (Figure 2b).

Table 1: Baseline characteristics of volunteers (mean±SEM)

Attributes	Fast finisher	Slow finisher	'p' value
Age (years)	23.9±0.6	24.5±0.6	0.46
Height (cm)	170.9±0.8	172.5±0.8	0.20
Weight (kg)	66.38±1.53	69.08±1.70	0.25
BMI (kg/m^2)	22.2±0.4	22.9±0.5	0.22
RHR (beats/min)	69.5±2.6	68.9±2.7	0.89
SBP (mm Hg)	120.0±3.3	128.3±3.0	0.08
DBP (mm Hg)	71.5±2.6	79.0±1.5	0.03*

SEM: Standard Error of Mean; p value: statistical significance (*= p<0.05); BMI: Body Mass Index; RHR: Resting Heart Rate; SBP: Systolic Blood Pressure; DBP: Diastolic Blood Pressure

Table 2: Cardio-respiratory responses of fast finisher and slow finisher during event (mean±SEM)

Attributes	Fast finisher	Slow finisher	'p' value
HR (beats/min)	180.9±2.12	181.5±1.34	0.81
BR (breath/min)	42.30±1.83	45.10±1.02	0.20
CBT (°C)	37.71±0.02	37.82±0.04	0.02*

SEM: Standard Error of Mean; p value: statistical significance (*= p<0.05); HR: Heart Rate during exercise; BR: Breathing rate during exercise; CBT: Core Body Temperature; °C: degree Celsius

During comparative analysis between BE2 vs AE2 of slow finisher found that the UCP1 ($p<0.001$) (Figure 2a), KYNA ($p=0.020$) (Figure 2b) and cortisol ($p=0.008$) (Figure 2e) levels were significantly higher in AE2; while SDH ($p<0.001$) (Figure 2d), BNP ($p=0.032$) (Figure 2f) and BDNF ($p=0.006$) (Figure 2g) levels were significantly lower in AE2 and no significant change was observed in level of BHB ($p=0.204$) (Figure 2c).

During comparative analysis of pre-exercise level of both groups (BE1 vs BE2), it was found that the KYNA ($p=0.013$) (Figure 2b), SDH ($p<0.001$) (Figure 2d) and BDNF ($p=0.015$) (Figure 2g) levels were significantly higher in BE2; while there were no significantly difference observed in UCP1 ($p=0.188$) (Figure 2a), BHB ($p=0.916$) (Figure 2c), cortisol ($p=0.71$) (Figure 2e) and BNP ($p=0.31$) (Figure 2f).

During comparative analysis of post-exercise level of both groups (AE1 vsAE2), it was observed that the UCP1 ($p=0.016$) (Figure 2a) and KYNA ($p<0.001$) (Figure 2b) levels were significantly higher in AE2; while BNP ($p<0.001$) (Figure 2f) was significantly lower in AE2 and there were no significantly differences were observed in BHB ($p=0.424$) (Figure 2c), SDH ($p=0.089$) (Figure 2d), cortisol ($p=0.169$) (Figure 2e) and BDNF ($p=0.278$) (Figure 2g).

DISCUSSION

The objective of this study was to quantify the cardio-respiratory and biochemical variables during military

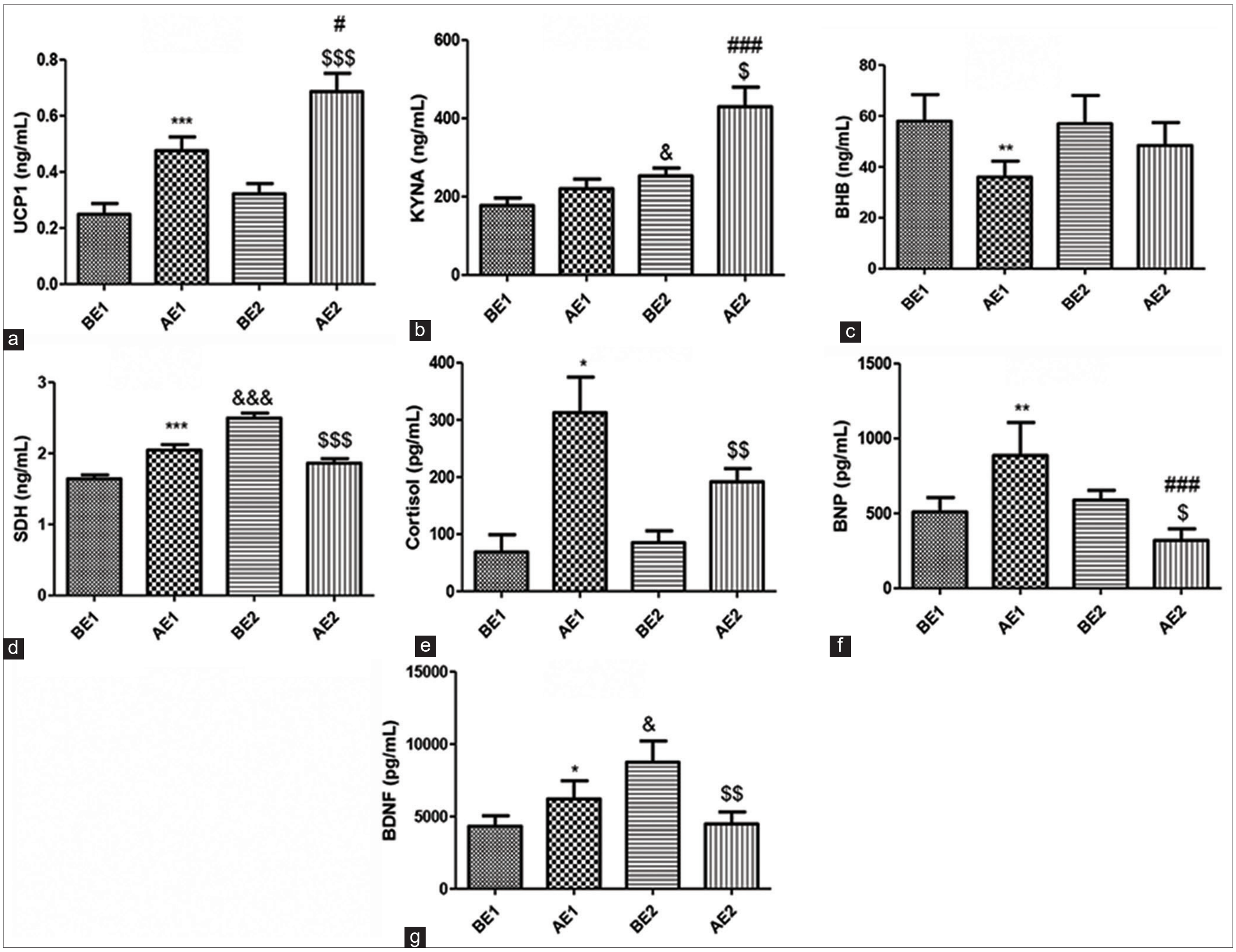

Figure 2: Graphical representation of biochemical responses of both groups before and after of the event (mean ± SEM). [Fig. 2(a): UCP1; 2(b): KYNA; 2(c): BHB; 2(d): SDH; 2(e): Cortisol; 2(f): BNP; 2(g): BDNF]
SEM: Standard Error of Mean; BE1: Response of Fast finisher before event; BE2: Response of Slow finisher before event; AE1: Response of Fast finisher after event; AE2: Response of Slow finisher after event. Symbol: (*: BE1 vs AE1); ($: BE2 vs AE2); (&: BE1 vs BE2); (#: AE1 vs AE2); (*, $, &, #: p value $\leq$ 0.05); (**, $$, &&, ##: p value $\leq$ 0.01); (***, $$$, &&&, ###: p value $\leq$ 0.001)

activity at jungle environment. During demographic assessment of all the participants, no significant differences were observed in their age, height, weight and BMI, but the DBP was significantly ($p < 0.03$) higher in fast finisher (Table 1). It was observed that the slow finisher performer has taken significantly more time ($p<0.0001$) in comparison to Fast finisher performer. In order to understand the cause behind the difference in time taken by both groups, their physiological responses were observed, it was found that the HR, BR and CBT was higher in slow finisher as compare to fast finisher (Table 2). Similar study was conducted by Bradbury et al. on soldiers in controlled condition and found that the rise in CBT was 38.5°C after 10.9 min of exercise, they had suggested that the rise in CBT normally observed during exercise is partly due to the proportional nature of the control mechanism and partly to the fall in skin temperature, which locally inhibits the heat dissipating responses.[23] Finding of Gleeson[7] was also in line to the suggestion of Bradbury that the lesser thermal gradients (difference between ambient temperature and skin temperature) may play crucial role in increasing the CBT.[23] The rise in CBT is directly proportional to the time dependent ability of individual to release heat into environment.[8] Bertholet et al. have mentioned that the UCP1 strongly contributes to the overall energy balance of performer.[24] UCP1 levels were measured in the present study, it was found that there was no significant difference in pre-exercise level of both groups, while significantly ($p<0.05$) higher in AE2 as compared to AE1 (Figure 2a). In present study the ambient temperatures (32-34°C) and higher humidity (75-90%) may cause additional thermal strain as the body also gains heat from the environment. The inability to dissipate metabolic heat generated during exercise might be due to lesser thermal gradient or higher rate of heat production with increased oxygen consumption in case of Slow finisher performer. It is notion that the muscular activity increased during extreme exercise causes an increase in heat production in the body due to the inefficient catabolism of carbohydrate and fat,[7] because the UCP1 may dissipate the energy stored in the proton motive force by mediating proton leak from the IMS to the matrix.[14] In present study, we observed KYNA level was significantly ($p<0.05$) higher in BE2 & AE2 as compare to BE1 & AE1 respectively while there was no significant difference was observed in case of BE1 vs AE1 (Figure 2b). Schlittler et al. have done study on cycling marathon and observed that the KYNA level was increased after exercise.[25] Increased KYNA level in plasma possibly depend on differences in the availability of free tryptophan (TRP). At rest, the majority of TRP in the blood is bound to albumin and only a small proportion is available in the free form. Free fatty acids (FFA) competitively bind to albumin, and dissociate the bound TRP thus increasing the concentration of free TRP in the blood.[13,26]

In skeletal muscle and liver, GPCR mediated activation of PKA increases glycogenolysis.[9] In the liver, PKA also stimulates gluconeogenesis through the transcriptional activation of numerous genes.[10] BHB are ketone bodies serve as a circulating energy source for tissues in times of fasting or prolonged exercise.[27] In present study, BHB was significantly decreased in AE1 as compared to BE1 while there were no significant changes observed in case of BE2vsAE2 and AE1vsAE2 (Figure 2c). Karl et al. conducted study on Norwegian Soldiers performing long duration endurance activity of 51 km, found that the level of BHB in plasma were significantly higher after exercise.[28] Sleiman et al. has reported that the BHB was significantly increased in hippocampus region after exercise,[29] which showed that BHB is transported in the blood stream to the brain where it can serve as an energy source.[30] It showed that the BHB synthesis would be relatively higher in long duration activity as compared to the short duration activity, because the BHB utilization rate could be higher. Thus, it may be reason that in our study, BHB utilization rate was significantly higher to fulfil the demand of energy in case of fast finisher as compare to slow finisher. Thus, the rate of gluconeogenesis could be higher in fast finisher as compared to slow finisher. SDH is reliable indicator of mitochondrial potential for ATP production.[12] In present study, it was observed that the SDH level in plasma were higher in AE1 as compared to BE1 while lower in AE2 as compared to BE2 (Figure 2d). This indicates the ATP production from stored energy source could be higher in Fast finisher performer as compared to slow finisher performer. Thus, the rate of glycogenolysis is relatively higher in fast finisher as compare to slow finisher. When the cortisol level was observed in our study, it was found that the cortisol was significantly higher in AE1 and AE2 with respect to BE1 and BE2 respectively (Figure 2e). Similar pattern of increment was observed by Leers et al.[17] Szivak et al. studied physiological stresses associated with 10 days long classified SERE training on US Navy men of similar age; it was observed that the cortisol increased significantly.[5] Kyröläinen et al. had conducted a study on Finnis Soldiers of similar age range; soldiers were undergone through training of 5-10 km for 5 days duration with 20-25 kg load on treadmill[31] and Merino et al. have conducted study on French Military of similar age range; soldiers undergone through five days of combat training course on real undulated terrain.[32] In both studies, it was observed that the cortisol levels were not significantly changed. It might be that the relatively short-term intensive physical activity leads to increased plasma levels of cortisol, as shown in the present study. A study on the relationship between exercise and cortisol showed that short-term medium to low strength exercises did not change plasma cortisol levels or may even have slightly reduced levels, whereas high-intensity exercise can elicit increase in blood plasma Cortisol levels.[18] This might be apparently beneficial for coping with the higher energy demand as a result of the acute stress during exercise.

BNP may release into the circulation as a counter-regulatory response for cardiorenal homeostasis to induce vasodilation.[15] The left ventricle is the predominant site of BNP synthesis in healthy men[33,34] and the plasma concentration of BNP increases in response to various degrees of ventricular overload.[35-37] Middleton et al. had concluded that the BNP level was significantly elevated after exercise.[38] In present study, we found that the BNP level was significantly higher in AE1 as compared to BE, and significantly decreased in AE2 as compared to BE2 (Figure 2f). In addition to this, it was observed that the DBP was significantly ($p<0.05$) higher in Slow finisher performer as compared to Fast finisher performer. Thus, the increased BNP level in fast finisher indicated that it could have more vasodilation capacity and less ventricular stress to disperse the heat generated during activity and have more capacity to tolerate higher ventricular workload.

Stress evaluations of participants in military mission are critically important and appropriate objective biological parameters that can evaluate stress are needed. In present study, BDNF was significantly higher in BE2 in comparison to BE1 while it was significantly ($p<0.05$) higher in AE1 in comparison to BE1 and significantly ($p<0.01$) lesser in AE2 in comparison to BE2 (Figure 2g). Plasma BDNF concentration more accurately reflects brain BDNF levels compared to serum BDNF because serum BDNF is predominantly derived from clotted peripheral platelets.[19] Numerous studies have reported that decreased levels of plasma BDNF in healthy subjects are associated with depression and psychological stress.[20,21,39,40] Previous study revealed that the BHB could promote the expression of BDNF during low glucose levels.[11] During fasting and extreme exercise, a shift of brain energy substrate utilization from glucose to the BHB occurs. During physical activity and resting condition, the brain contributes to approximately 70% - 80% of the circulating BDNF levels, and thus acts as its main source. However, other peripheral sources such as platelets, vascular endothelial cells, and skeletal muscles also contribute to the BDNF.[41] BDNF can cross the blood-brain barrier in both directions (Brain to the periphery and vice-versa). The increase in the BDNF levels after the acute physical activity can be due to its enhanced release from different tissues including the active muscles and brain. Also, the increase in its levels after the chronic training may be caused by the increase in gene expression and activating the transcription pathways. Therefore, increase in the BDNF levels, aside from its roles in neurogenesis and brain health, plays a critical role in central and peripheral energy metabolism through hypothalamus pathways.[42]

CONCLUSION

The mechanisms responsible for these changes in performance are complex, as changes in core body temperature would alter a range of physiological factors including the metabolic, cardiovascular, and thermoregulatory response to exercise. It is therefore possible that any of these changes, either in isolation or combination, would impact upon the ability of a soldier to perform work. The outcome of this study may indicate that the slow finishers were more susceptible to risk of injury, because the exercise induced thermogenesis and mental stress were higher in comparison to fast finisher. Finding of this study may be helpful in understanding the metabolic demand of individuals undergoing through such strenuous events and making strategies to reduce risk of injury and enhance performance during extreme military training event.

Limitation of study

Present study was done on smaller number of healthy male soldiers in jungle environment. Physiological study in detailed with greater sample size is needed.

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Author's contribution:
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