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Impact of allostatic load on hormone status in special-forces operators

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Abstract

To date, there is limited data on the hormone responses that occur during intensive operational training in U.S. Special Forces Operators (SFO). Therefore, the purpose of this investigation is to: (1) quantify hormone profiles of SFO, and (2) examine hormone responses that occur following 5 days of Winter Warfare Training (WWT) in U.S. Army SFO. Blood samples were collected at baseline, immediately pre-WWT, and post-WWT. Reductions in both total and free testosterone were observed from baseline to pre-WWT ($p<0.001$), and from pre-WWT to post-WWT ($p<0.001$). The total testosterone to cortisol ratio decreased from baseline to pre-WWT ($p<0.001$) and from pre- to post-WWT (p=0.002).Thyroid hormones were elevated pre-WWT relative to baseline ($p<0.05$). Leptin decreased from baseline to pre-WWT ($p<0.001$) and from pre- to post-WWT ($p<0.001$). Our data demonstrates heightened physiological strain both prior to and following WWT, as exhibited by changes in growth-related, stress, and metabolic hormones.

Keywords: Special forces operators, winter warfare training, allostatic load, hormones, stress

Introduction

Special Forces Operators (SFO) are highly trained military personnel known for their ability to operate independently and/or in small teams to execute high-risk missions. U.S. Army Special Forces Group (SFG) Operational Detachment Alphas (ODA) operate in small tactical teams and have received specialized training in advanced weapons, language, demolitions, combat medicine, military free-fall, and advanced combat tactics. During training and in combat, operators are exposed to a variety of stressors, such as sleep deprivation, increased physical demand, and environmental extremes associated with temperature, terrain, and weather. While stress can promote adaptation, chronic and/or frequent exposure to stress, as experienced by military personnel, can negatively impact performance [1] and long-term health [2].

The physiological burden that accrues when stress load exceeds the body's ability to optimally cope (i.e., allostatic load) can disrupt several neuroendocrine systems, including the hypothalamic-pituitary adrenal axis (HPAA), HP gonadal axis (HPGA), and HP thyroid axis (HPTA) [3]. The HPAA consists of a system of hormones that work synergistically to regulate the body's stress response, HPGA hormones maintain reproductive health and fertility, and the HPTA is primarily responsible for metabolic regulation [2]. Stress impacts all three interconnected neuroendocrine systems, which is observed via alterations in the stress, sex, and metabolic hormones that are involved in these pathways [4, 5, 6-10]. An increase in physiological demand will render shifts in energy distribution towards metabolic processes involved in coping with the increased workload and away from reproductive function and other anabolic mechanisms that are not geared towards survival [11]. For example, increases in cortisol (primary stress hormone) and reductions in anabolic hormones, namely total testosterone (TT), free testosterone (FT), and insulin-like growth factor 1 (IGF-1) have been observed during intensive military trainings [4, 6, 7, 9]. Furthermore, stress-induced disruptions in hormone milieu are associated with reductions in both cognitive and physical performance, as well as heightened injury risk in military operators [4]. Therefore, it is critical to gain more knowledge on the impacts of chronic stress exposure on operator physiology and capacity to cope with allostatic load.

To date, a majority of military studies have focused on performance and injury risk in conventional military forces [6, 7, 12], while few have sought to characterize hormone profiles of more experienced operators during operational trainings [5, 13]. Operational trainings equip SFO with specialized tactical skill sets critical for executing high-risk missions in extreme environments. While conventional training prepares inexperienced military personnel for the physical and operational demands endured during conventional warfare. In a previous study that investigated the impact of training location on operator physiology, we showed alterations in several hormones and binding proteins, most notably changes in stress and anabolic hormones, as well as impaired cognitive performance in U.S. Army Soldiers during operational training evolutions [5].

Given the association between hormonal dysfunction, impaired performance, and heightened injury risk, it is critical to enhance our understanding of: (1) the hormone profiles of elite military populations, and (2) the capacity of operators to withstand the allostatic load experienced during training and in combat. Therefore, the purpose of this study is to quantify endocrine profiles and capture the hormone responses that occur during Winter Warfare Training (WWT), operational training that prepares SFO to move effectively in arduous environments (i.e., harsh terrain, severe weather, and/or extreme temperatures) as they master critical skills, such as alpine skiing, snow-machine, skinning, and marksmanship. Knowledge on how operating in multi-stressor environments impacts operator physiology is imperative for optimizing their readiness and performance during both training and in combat, as well as long-term health.

Materials and Methods

Participant Recruitment

Seven male active-duty U.S. Army SFO stationed in Joint Base Lewis-McChord (JBLM), Washington (WA), USA, volunteered to participate in this study. Written and verbal consent was obtained from all participants by research staff prior to training. The study protocol (NHRC.2020.0004) was approved by the Naval Health Research Center Institutional Review Board in compliance with all applicable federal regulations governing the protection of human subjects.

Baseline Assessment

Participants' anthropometric measurements (i.e., height [cm.], weight [kg]) and body fat percentage (BF%) were assessed in the morning between 0700-0800. Height and weight were measured with a stadiometer to the nearest 0.1 cm (SECA North America, Chino, California [CA], USA) and SECA bodyweight scale to the nearest 0.1 kg (SECA North America, Chino, CA, USA). BF% was determined using a dual x-ray absorptiometry (DXA) machine (Lunar Prodigy, GE Healthcare, Madison, Wisconsin, USA). Maximal oxygen consumption ($VO_{2\ max}$) was measured with a metabolic cart (Quark RMR, COSMED USA Inc., CA, USA) while performing a modified Bruce Treadmill Protocol, as previously described [14].

Winter Warfare Training

WWT took place in the Pacific Northwest area of WA in March 2023. WWT was a 5-day unsupported and unconventional movement in which each participant carried their own food, water, and supplies for the duration of training. Training consisted of a 5-day movement on skis over mountainous terrain and participants slept in tents over the duration of the training. Average daily temperatures ranged between 23°F (night) to 37.9°F (day) and terrain consisted of heavy snow.

Physiological monitoring

Participants wore Polar Grit X physiological monitors (Polar® Grit X Pro, Polar Electro USA, Lake Success, New York, USA) during VO_2 max testing and throughout WWT to capture heart rate (HR; beats per minute [bpm]), estimated energy expenditure (via HR; kilocalories [kcal]), sleep duration, and daily workload. The Polar Grit X Pro was selected based on internal validation [14] and prior validation of the Polar Vantage for tracking steps, acceptability among military personnel, ease of use, efficient battery life, and feasibility [15, 16]. Wearable devices were programmed with individual participant information (i.e., age, height, weight) for native algorithms that rely on this information for prediction of outcome metrics. Participants were asked to wear devices on their non-dominant hand to ensure the most accurate data per manufacturer recommendations. Daily active time was determined via an internal 3D accelerometer that records wrist movements and analyzes the frequency, intensity and regularity of movements together with physical information, allowing a summation of daily activity coupled with that of regular training. Participants captured activity specific to training (i.e., exclusion of non-training movement) by "starting" and "stopping" and "saving" an activity on their Polar Grit X device. Oxygen uptake (VO_2) during training was estimated by comparing HR during the recorded activity to VO_2 at the same HR from the participant's VO_2 max test. Physical activity intensities were classified based on American College of Sports Medicine (ACSM) guidelines [17]. Basal metabolic rate (BMR) was calculated using the Mifflin-St Jeor equation for men: 10 x weight (kg) + 6.25 x height (cm) – 5 x age (years) + 5 [18].

Blood Sampling

Fasting whole blood (10 mL) was collected via venipuncture of the antecubital area with ethylenediaminetetraacetic acid (EDTA) collection tubes. Samples were collected in the morning (Between 0700-0800) at their home station in WA prior to WWT (baseline), and on the mornings (between 0700-0830) of the first (day 1 [pre]) and last day (day 5 [post]) of training. Blood samples were centrifuged at 3,000 RPM (1,000 x g, 4 °C) for 15 minutes. Plasma was immediately aliquoted into 2 mL cryogenic storage vials (Corning Inc., Corning, NY, USA) and placed on dry ice prior to placement in a -80 °C freezer until analysis.

Plasma Analysis

All plasma samples were analyzed for the following biomarkers via enzyme-linked immunosorbent assay (ELISA): adrenocorticotropic hormone (ACTH), cortisol, TT, FT, dehydroepiandrosterone-sulfate (DHEA-S), thyroid stimulating hormone (TSH), Triiodothyronine (T_3), Thyroxine (T_4; Calbiotech, El Cajon, CA, USA), IGF-1, leptin, insulin-like growth factor binding protein 3 (IGFBP-3), and sex hormone binding globulin (SHBG; Abcam, Cambridge, MA, USA). A BioTek Synergy 2 Plate Reader Multi-Mode (Agilent, Santa Clara, CA, USA) was used to determine the optical density of each well at an absorbance of 450 nm. Biomarker concentrations were determined with MyAssays Online Limited (Brighton, England, UK) using Four and Five Parameter Logistic (4PL; 5PL) Regression models.

Statistical Analysis

Statistical analyses were performed using R.4.2.1 (R Core Team, 2022) and the *Rallfun-v41* package as described [19]. To assess differences in hormones baseline to pre-WWT, and pre- and post-WWT, pairwise comparisons of the means were performed using a percentile bootstrap method. Hochberg's method was employed to control the false discovery rate. A p-value of <0.05 was regarded as statistically significant. All data is represented as means± standard error of the mean (SEM).

Results

Participant Characteristics

Seven male U.S. Army ODA operators (age: 33±1.0 yrs., height: 179.0±1.5 cm, weight: 85.5±1.9 kg, BF%: 16.2±1.0%, $VO_{2\,max}$: 45.3±1.6 ml/kg/min, BMR: 1814±26 kcal/day) completed this study.

Energy Expenditure, Workload, and Sleep

Participants spent an average of 5.5 hours per day performing training activities, including backcountry skiing and mountaineering. In total, operators completed approximately 28 hours of physical movement during five days of WWT. Specifically, 26 hours were spent doing vigorous intensity activity (>60% VO_2 max) while moderate intensity activity (40-60% VO_2 max) comprised 2 hours of total training time. Energy expenditure (EE) averaged 5265 kcals/day (range: 2919-7574 kcals/day), and total daily workload (estimated mileage from steps) averaged 12.0 miles per day (29,916 steps per day), totaling 61.4 miles. Average daily active time was 11 hours and 21 minutes; operators slept between 4 and 9 hours per night. Operators' physiological measures by training day appear in Table 1.

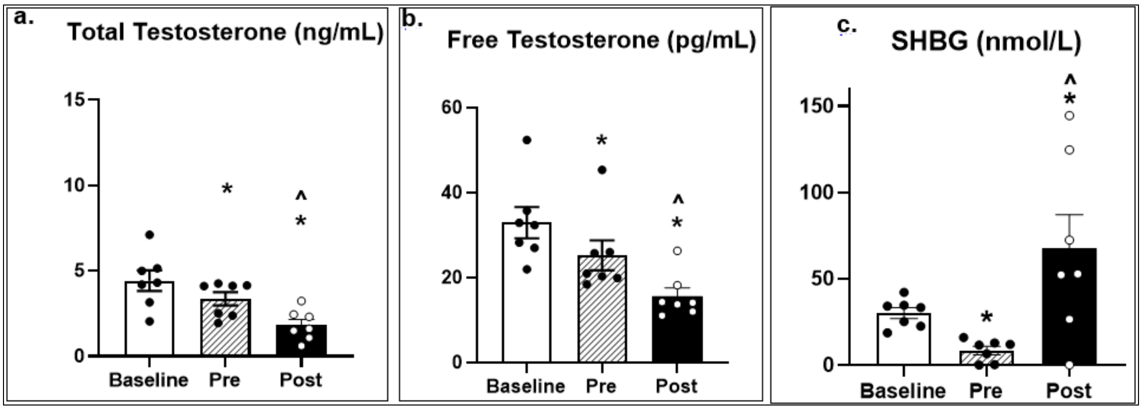
Table 1: SFO Physiology by Training Day

Physiological measure	Day 1	Day 2	Day 3	Day 4	Day 5
EE (kcals)					
Exercise EE	2959±582	901±107	4916±323	3336±668	1738±314
Total Daily EE	6225±381	4008±325	6577±628	6189±467	3247±362
Workload					
Training Duration (hr: min)	5:23±0:52	2:07±0:00	9:41±0.06	6:41±1:21	3:06±0:05
Distance (mi)	16.3±2.4	5.7±0.9	17.4±3.8	15.5±2.2	6.6±0.5
%$VO_{2\,max}$	60%±4%	45%±19%	64%±8%	60%±5%	55%±7%
Average HR (bpm)	124±2	109±4	123±4	120±1	121±8
MHR (bpm)	169±3	151±8	169±5	164±3	155±4
METS	7.6±0.4	5.5±1.5	7.8±0.3	7.7±0.5	7.4±0.8
Sleep					
Duration (hr: min)	-	5:54±0.17	7:53±0:12	7:21±0.50	7:35±0:37
Average HR (bpm)	-	56±2	53±3	51±4	50±2

All data is represented as mean ±standard error of the mean (SEM; n=7). SFO = Special Forces Operators; EE = energy expenditure; kcals = kilocalories; hr. = hours; min = minutes; mi = miles; %VO2 max = percentage of maximal oxygen consumption; HR = heart rate; bmp = beats per minute; MHR = maximum heart rate; METS = metabolic equivalents. Sleep data was not captured Day 1, as training began that evening and participants did not sleep.

3.3 Growth-Related Hormones

Pairwise comparisons revealed significant changes in TT, FT, and SHBG levels during WWT $p<0.001$; Figure 1a-f). TT decreased by 23% from baseline (4.4±0.6 ng/mL) to pre-WWT (3.5±0.4 ng/mL; $p<0.001$), and by 47% from pre- to post-WWT (1.8±0.3 ng/mL; $p<0.001$; Figure 1a). Similarly, FT declined by 19.2% from baseline (31.2±3.7 pg/mL) to pre-WWT (25.2±3.5 pg/mL), and by 38% from pre- to post-WWT (15.6±2.0 pg/mL; $p<0.001$; Figure 1b). A concomitant 72% decrease in SHBG was observed from baseline (30.2±0.1 nmol/L) to pre-WWT (8.5±2.4 nmol/L; $p<0.001$), while levels increased by 696% from pre- to post-WWT (67.7±19.5 nmol/L; p=0.006; Figure 1c).



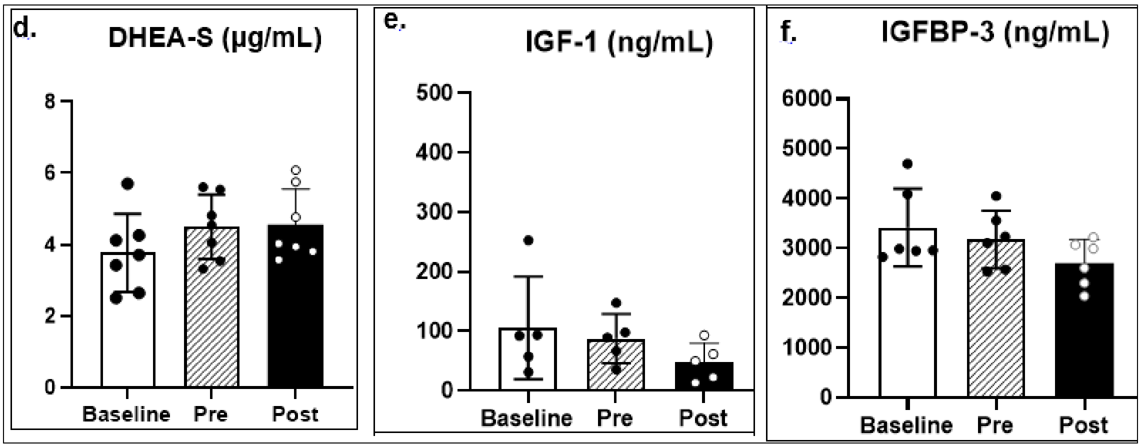


Fig 1: *Growth-related hormones (mean± SEM) at baseline, pre-, and post-WWT.* Plasma concentrations of (a) total testosterone (n=7), (b) free testosterone (n=7), (c) sex hormone binding globulin (SHBG; n=7), (d) dehydroepiandrosterone-sulfate (DHEA-S; n=7), (e) insulin-like growth factor 1 (IGF-1; n=5), and (f) insulin-like growth factor binding protein 3 (IGFBP-3; n=6). (*) significantly different from baseline ($p<0.001$). (^) significantly different from pre ($p<0.05$). Each circle represents one participant's value, and the bars represent the mean concentration for each time point (n=7).

3.4 Stress Biomarkers

Pairwise comparisons revealed significant changes in biomarkers related to stress during WWT (Figure 2a-c; $p<0.05$). Plasma ACTH decreased by 63% from pre- (23.9±4.0 pg/mL) to post-WWT (8.8±1.7 pg/mL; $p<0.001$; Figure 2a). While no differences were observed in cortisol from pre- to post-WWT, pre levels (145.5±28.6 ng/mL) were 152% higher than those observed for baseline (57.8±4.5 ng/mL; $p<0.001$; Figure 2b). The TT: C (molar ratio) decreased by 30% from baseline (0.1±0.02) to pre-WWT (0.04±0.0006; $p<0.001$), and by an additional 50% from pre-WWT levels to post (0.02±0.006; p=0.002; Figure 2c).

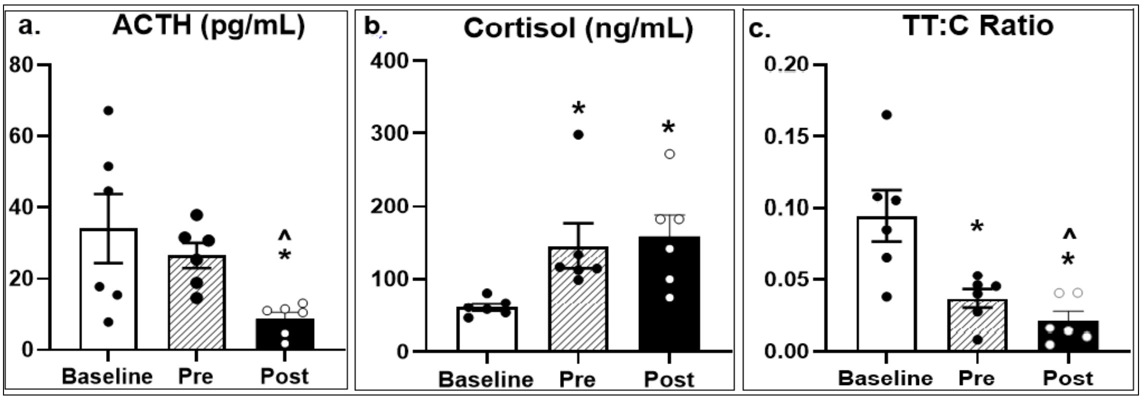


Fig 2: *Stress hormones and the total testosterone: cortisol ratio (TT:C; mean±SEM) at baseline, pre-, and post-WWT.* Plasma concentrations of (a) adrenocorticotropic hormone (ACTH), (b) cortisol, and (c) the TT:C (molar ratio). (*) significantly different from baseline ($p<0.001$). (^) significantly different from pre ($p<0.05$). Each circle represents one participant's value, and the bars represent the mean concentration for each time point (n=6).

Metabolic Hormones

Interestingly, changes in TSH, T_3, and T_4, were observed from baseline to pre-WWT ($p<0.05$; Figure 3a-d); however, no alterations in these hormones were observed between pre- and post-WWT. More specifically, TSH was 282% higher during pre-WWT (5366±483 µIU/L) relative to baseline (1404.7±22.6 µIU/L; $p<0.001$; Figure 3a), T_3 was 13% higher during pre-WWT (133.4±7.8 µIU/L) compared to baseline (117.6±9.7 µIU/L; p=0.006; Figure 3b), and T_4 increased by 61% from baseline (5.9±0.5 µg/dL) to pre-WWT (9.5±0.9 µg/dL; $p<0.001$; Figure 3c). Leptin decreased by 15% from baseline (16.4±4.7 ng/mL) to pre-WWT (13.9±5.0 ng/mL; $p<0.001$), and by an additional 89% from pre- to post-WWT (1.5±1.1 ng/mL; $p<0.001$; Figure 3d).

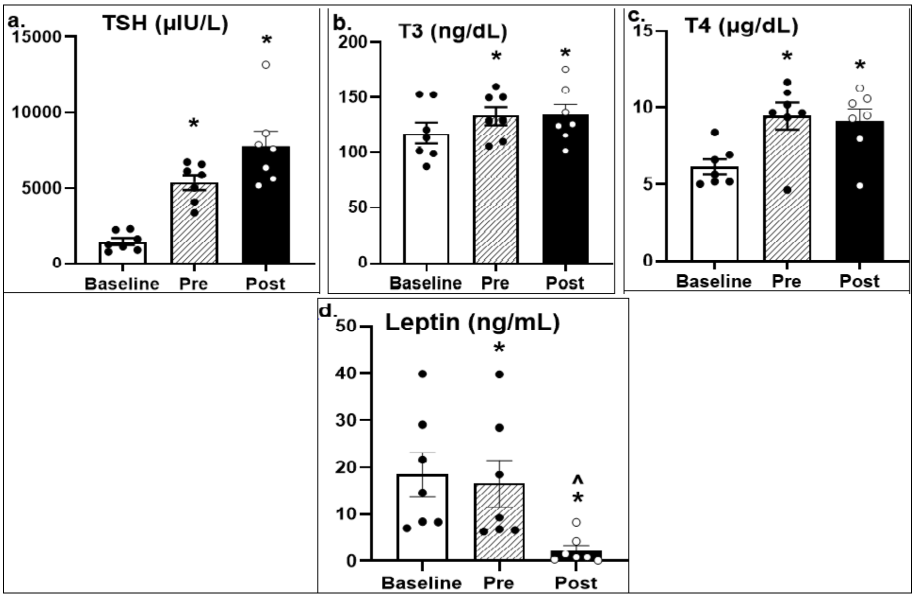


Fig 3: *Metabolic hormones (mean±SEM) at baseline, pre-, and post-WWT.* Plasma concentrations of (a) thyroid stimulating hormone (TSH), (b) triiodothyronine (T$_3$), (c) thyroxine (T$_4$), and (d) leptin. (*) significantly different from baseline ($p<0.05$). (^) significantly different from pre ($p<0.05$). Each circle represents one participant's value, and the bars represent the mean concentration for each time point (n=7).

Discussion

Our findings demonstrate alterations in growth, stress, and metabolic hormones in SFO prior to and following WWT. Notable decrements in TT and FT occurred from baseline to pre-WWT and, further, from pre- to post-WWT. Additionally, SHBG levels increased significantly (696%) from pre- to post-WWT. Training-induced changes in growth hormones, such as TT, FT, and SHBG, have been well documented during strenuous military trainings [1, 4, 6, 7, 19, 19]. Shifts in energy balance and expenditure, resulting from heightened physical activity, insufficient caloric intake, and an increase in thermogenesis, will promote the re-allocation of energy from biological processes, such as reproductive function and systemic anabolic activity, to those aimed at meeting the physiological demands of an increased workload [21]. In line with previous data [1, 6, 7, 10], we observed significant reductions in TT and FT and a concomitant increase in SHBG, which was paralleled by a decrease in TT:C, a well-established proxy for physiological strain in military populations [7]. Likewise, we documented similar changes in TT and the TT:C ratio in a separate group of U.S. Army SFO ODAs during WWT [5]. Furthermore, findings from both studies align with those of Hamarsland *et al*., which demonstrated marked hormone dysregulation in Norwegian SFOs during and following hell week [22].

In addition to changes in growth-related hormones from pre- to post-WWT, reductions in TT, FT, and TT:C ratio were seen between time points prior to training (i.e., baseline and pre-WWT), which may stem from variability in environmental stressors, such as temperature, and terrain, between the operators' home station (JBLM, WA; altitude: 119-292 meters [m]; temp: 52°-57°F) and the Cascade Mountain Range, namely Mount Rainer, in WA (altitude: 4,392 m; temp: 24°-38°F), which had heavy snow during WWT. Likewise, in our previous study [5], we observed a substantial reduction (42%) in the TT: C ratio from baseline (Fort Carson, Colorado [CO]) to pre-WWT (Montana [MT]) in operators. Our data are further supported by Hamarsland *et al*., 2018, who reported a decrease in FT from baseline to pre-hell week [22]. Our results, coupled with findings from prior investigations, suggest that non-training stressors disrupt the SFO's hormone milieu, which may have a compounding effect on the physical stress experienced by operators during military trainings and lead to allostatic load.

Moreover, alterations in metabolic hormones (TSH, T$_3$, T$_4$, and leptin) were demonstrated throughout the duration of the current study. Specifically, marked increases in TSH, T$_3$, T$_4$ and a concurrent decrease in leptin were observed between baseline and pre-WWT in operators. While these results contradict those observed between pre-WWT time points (i.e., baseline and pre-WWT) in our previous study [5], the lack of training-induced differences (pre- to post-WWT) in metabolic hormones is similar between the two investigations. The absence of training-induced changes in thyroid hormones in these two warfighter populations may be suggestive of metabolic adaptation [23]. Metabolism is, in part, regulated by thyroid hormones [24]; therefore, the lack of a training-induced response could indicate that operators were in a state of caloric balance during WWT, despite increases in exercise EE [25]. However, caloric intake was not assessed in this current study, thus, the validity of this explanation remains speculative. Additionally, the significant decline in leptin observed between baseline and pre-WWT and pre-WWT and post-WWT, may be related to alterations in fuel regulation, BMR, and/or BF% (RG, M & DA, J 2003). Changes in all metabolic hormones (TSH, T$_3$, T$_4$, and leptin) were seen from baseline to pre-WWT, which may stem from an increase in thermogenesis, resulting from lower temperatures and snowy conditions during WWT.

Limitations

This study contains several limitations. First, the sample size is limited and there is no comparative control group due to the nature and type of training. The participants are highly fit military personnel (VO$_2$ max: 45.3±1.6 ml/kg/min) accustomed to training in arduous environments, whereby, frequent cold exposure is associated with physiological adaptation [23]. Also, operators grew up in different locations throughout the U.S. and vary in the time they have spent in the service. The impact of nutrition was not examined, as operators were "free-living" and had autonomy over dietary intake. Additionally, blood samples were acquired at only 3 time-points. This limits our understanding of daily circulating hormone levels during WWT and the time course of hormonal modification. Lastly, baseline sampling location was not uniform among operators and was taken ~ 7.5-10 weeks prior to WWT due to logistical reasons, which may have impacted our results.

Conclusion

To date, there is limited information on the physiological impact of operating/training in multi-stressor environments on elite military operators. A majority of military studies have investigated disruptions in hormone milieu in less experienced, non-SFO populations, whereas we captured hormone responses in U.S. Army SFO during a training evolution which specifically prepares elite warfighters to execute high-risk missions in extreme environments. Our data demonstrates heightened physiological strain both prior to and following WWT, as exhibited by changes in growth-related, stress, and metabolic hormones. Disruptions in hormone levels during intensive military trainings are expected due to increased physiological demands. The lack of disturbances exhibited in thyroid hormones (TSH, T_3, and T_4) in SFO may be suggestive of metabolic adaptation, as these individuals are accustomed to training under harsh environmental conditions. Interestingly, several hormone levels were altered from baseline to pre-WWT, suggesting that non-training related stressors such as, environment, sleep, and/or diet, could have negatively influenced operator physiology. Being in a suboptimal physiological state at the onset of operational training could heighten injury risk, and compromise warfighter readiness, performance, and potentially long-term health. Therefore, incorporating an acclimatization period into operational trainings and allowing for optimal recovery time may benefit operator's health and success. Gaining greater insight on operator hormone status prior to and following operational trainings could be advantageous for scheduling further training evolutions and missions, as well as optimizing warfighter preparedness, performance, and health. Future research is warranted on: (1) duration of hormone recovery in elite operators, (2) physiological adaptation to allostatic load, and (3) the chronic impact of frequent stress exposure on operator health.

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