



BRIEF REPORT

Sleep Loss During Military Training Reduces Testosterone in U.S. Army Rangers: A Two-Study Series

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Abstract

Background: U.S. Army Rangers must maintain a high level of physical fitness in order to be successful during training and deployment missions. The hormone testosterone increases muscle growth and strength, which leads to better physical fitness and performance. Therefore, maintaining high testosterone levels is of critical importance to this unit. However, many Ranger missions require sleep deprivation, which, in non-military populations, has been shown to decrease testosterone. The purpose of the study was to test the impact of sleep deprivation during military training on U.S. Army Ranger testosterone levels.

Methods: In two studies surrounding Ranger training missions, salivary testosterone was measured within subjects during a rested condition and following sleep loss in male participants (Study 1 $n = 76$ males; Study 2 $n = 44$ males). Testosterone levels were compared using paired-sample t -tests. Additionally, given the diurnal secretion of testosterone, we compared testosterone levels assessed after sleep deprivation to historical data measured at the same time of day in participants who were well-rested.

Results: In Study 1, testosterone significantly decreased from 105.91 ± 35.35 to 79.87 ± 35.65 pg/mL (a 28% reduction) after sleep deprivation ($t(75) = 5.85$, $p = 0.001$). Similarly, in Study 2, following sleep deprivation, testosterone significantly decreased from 115.31 ± 28.44 to 89.29 ± 29.00 pg/mL (a 25.4% reduction; $t(43) = 4.81$, $p = 0.001$). In addition, Ranger testosterone was roughly 90% lower than historical data sampled at the same time of day.

Conclusion: These data add to the growing body of literature showing that loss of sleep decreases testosterone, which may ultimately have a negative impact on physical training and performance in physically elite populations like the U.S. Army Rangers.

Keywords

Military, Testosterone, Sleep deprivation, Rangers, Elite athletes

Abbreviations

pg/mL = picograms per milliliter

Introduction

Testosterone is an anabolic hormone that promotes protein synthesis, supports muscle hypertrophy/growth, and increases strength [1]. Higher levels of testosterone have been associated with better physical performance, while lower levels of testosterone have been linked with blunted protein synthesis [2]. The 75th Ranger Regiment, an elite special operations unit that is considered the premier raid force of the U.S. Army, must often perform physical challenges to execute missions successfully. Accordingly, physical fitness and maintaining high testosterone levels are critical priorities for this unit so that Rangers can perform well during exercise, training missions, and combat operations.

Several studies have shown testosterone decreases over the course of extended military training events (see review [1]). For instance, Nindl, et al. found an 80% decrease in testosterone over an 8-week leadership and tactical skill training course that all Rangers must attend [3]. Similarly, an additional study found a 50% decrease in testosterone after a 3-week specialized survival training course, which many special operations soldiers, including Rangers, take part in [4]. Such dips in testosterone are not inconsequential, as physical performance also tends to decline over the course of these training events (see review [5]). It is possible that reductions in testosterone, in conjunction with other physiological changes (e.g., decreased growth hormone or increased cortisol), contributed to a reduction in performance. However, in those studies, testosterone was tested before and after several weeks or months of physically demanding challenges. Therefore, the authors of the reported findings could only speculate about the specific factors that impacted testosterone throughout the extended training events.

A recent systematic review proposed that sleep loss, which is pervasive during military training missions, may be a key mechanism that decreases testosterone during these events [1]. There is a well-established link between sleep and testosterone in the general population. In the morning, testosterone is high, and it decreases linearly with time spent awake [6]. Sleep - not simply a period of rest - then actively restores testosterone to high morning levels. Disturbed sleep (or a lack of sleep) prevents the testosterone reset from occurring, thus preventing a high testosterone morning peak and reducing testosterone throughout the day [7,8,9]. Despite the probable link between military training, sleep, and testosterone, there has been no published investigation of this hypothesis. The current study series aimed to fill this gap by measuring salivary testosterone during a Ranger training mission before and after a single night of total sleep deprivation. By doing so, the current study aimed to rule out other additional factors (e.g., weeks or months of physical stress and caloric restriction) that may impact testosterone during more extended military missions.

Further, we compared these data to testosterone levels sampled before and after a restorative night of sleep in a comparable athlete population to determine the extent to which sleep loss impacted testosterone in

the current sample. It was hypothesized Rangers would have significantly lower testosterone following sleep loss than during baseline (prior to sleep loss). It was also hypothesized Rangers would have lower testosterone during the morning hours (when testosterone should be high) relative to the well-slept historical sample. The findings have important implications for both elite Soldiers and elite athletes who must undergo prolonged physical training or competition without adequate restorative sleep.

Methods

Testosterone levels were obtained during two observational studies assessing the impact of sleep loss on cognitive and physical performance during routine Ranger training missions. Rangers were recruited from two separate companies (units of roughly 150 soldiers) for each study. In order to maximize ecological relevance, convenience sampling (also known as availability sampling) was used, meaning any soldier who wanted to participate was enrolled, if over the age of 18. This type of sampling is appropriate when the researchers do not wish to generalize results to broader populations (e.g., populations beyond elite soldiers or athletes) [10]. Participants provided written informed consent prior to participation. All methods and procedures are in accordance with the Declaration of Helsinki. The study was approved by the Walter Reed Army Institute of Research Institutional Review Board and the United States Army Special Operations Command Research Advisory Committee.

Ranger demographics and study/training mission differences are presented in Table 1. All participants were male and similarly aged in both studies. There was a high amount of attrition due to the ecological nature of data collection during a training exercise (i.e., some individuals had essential duties to perform and were unable to provide a sample).

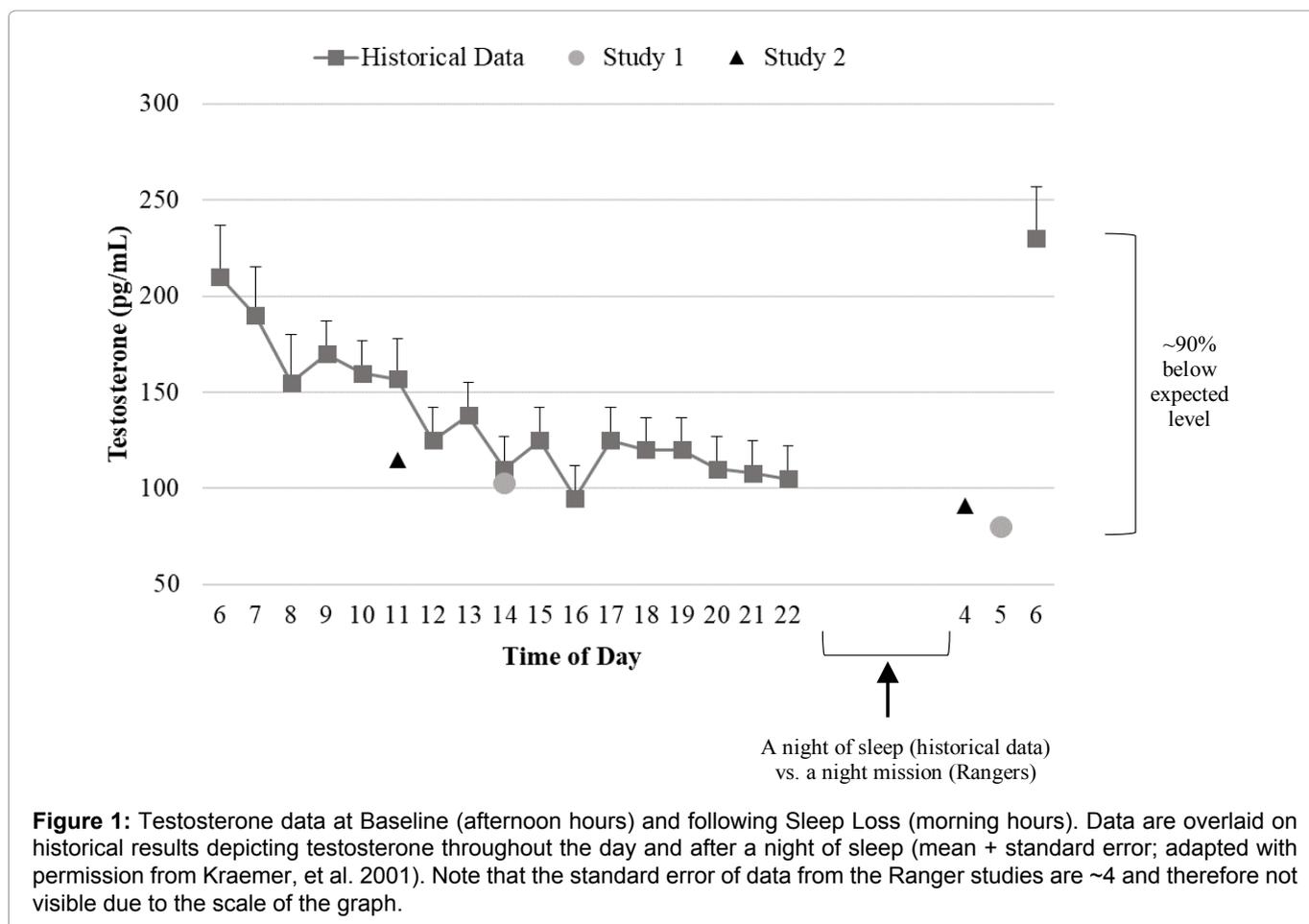
During both studies, salivary testosterone (described below) was sampled at a rested baseline condition and following the training mission in a sleep restricted state. The initial levels of testosterone ("Baseline") were sampled in the afternoon during a standard duty day (i.e., not undergoing training), when Rangers were sleeping on a normal nocturnal schedule. Time point 2 ("Sleep Loss") levels were sampled immediately after Rangers

Table 1: Demographics and study details. Numbers in parentheses indicate characteristics for soldiers who provided samples at both time points.

	Study 1	Study 2
<i>n</i>	103 (76)	74 (45)
Age in years	25.69 ± 4.09 (25.95 ± 4.00)	23.48 ± 3.90 (24.00 ± 3.14)
Baseline saliva sampling time	1400	1100
Sleep Loss saliva sampling time	0500	0400
Training activities	Paratrooper preparation	Ground assault preparation

Table 2: Testosterone values (expressed as pg/mL) from Study 1 and Study 2. SD = standard deviation. 95%CI = 95% confidence interval.

	Study 1			Study 2		
	Mean	SD	95%CI	Mean	SD	95%CI
Baseline	105.91	35.35	99.00, 112.82	115.31	28.44	108.72, 121.90
Sleep Loss	79.87	35.65	71.77, 87.85	89.29	29.00	80.58, 98.00



completed their first mission of the training cycle (i.e., after a full night of sleep loss).

Salivary testosterone

Saliva was sampled by the passive drool method [9]. After Rangers completed their mission, they vigorously swished their mouth with water to remove debris and were instructed to not ingest food or liquid for 10 minutes. Participants then provided 1 mL of saliva. Salivary free testosterone was quantified using the Salimetrics (Carlsbad, CA) Salivary Testosterone Enzyme Immunoassay Kit. Samples were tested in duplicate, and the average of the two values was used for analysis. Three statistical outliers (± 3 standard deviations of the mean) were removed from Study 1, and 1 outlier was removed from Study 2. Testosterone is expressed as pg/mL.

Statistical analyses

Paired-sample *t*-tests compared within-subject testosterone levels between Baseline and Sleep Loss. A descriptive comparison was then conducted between the testosterone levels measured in this study and those

from a previous in-lab study, which assessed how sleep resets testosterone during the night [6].

Results

Testosterone values are presented in Table 2. As hypothesized, for Study 1, there was a significant reduction in testosterone between Baseline and Sleep Loss ($t(75) = 5.85, p = 0.001$; a 28.0% reduction). For Study 2, there was also a similar significant reduction between Baseline and Sleep Loss ($t(43) = 4.81, p = 0.001$; 25.4% reduction).

As shown in Figure 1, although Baseline testosterone was in the expected range (compared to historical values), Ranger Sleep Loss testosterone values were roughly 90% lower than expected at the time of the morning testosterone peak.

Discussion

In two naturalistic studies of U.S. Army Rangers, testosterone was significantly lower after a nocturnal training mission with no sleep than during a previous

well-rested time point. These data replicate findings that testosterone decreases during military training [2-4] and expands the literature by demonstrating a single night of nocturnal training - with associated sleep deprivation - can reduce testosterone by 25-30%. We demonstrated a reduction in testosterone after the first mission night of two separate training events (during which Rangers were completing different missions), indicating the reduction in testosterone was not due to specific training activities. Rather, we believe the reduction in testosterone detected here was due to sleep deprivation - the common element between training events. Additionally, the extent of the testosterone reduction became clearer when these values were plotted against historical testosterone values from a demographically-similar population [6]. Ranger testosterone was strikingly lower during the morning (after training) than at a comparable time of day in the historical data. The latter finding may indicate that the expected testosterone rise that occurs during sleep is disrupted by these training events.

These data provide insight into a potential mechanism linking sleep and physical training/performance. Several studies have noted sleep loss leads to a decrease in upper and lower limb performance and increased exercise-related injuries (see review [11]). Additionally, our team previously found that sleep "extension" (which is achieved by purposefully sleeping more than usual) can improve standing long jump distance in a sample of college tactical athletes [12]. Sleep extension has also been found to provide cognitive resilience during subsequent sleep loss and leads to faster cognitive recovery following sleep loss [13]. Therefore, for physical performance, sleep extension may similarly provide resilience against and recovery from sleep loss that occurs during military or athletic training. In conclusion, although the physiological mechanism linking sleep and performance has yet to be fully established, we believe testosterone is a viable link between these two factors. Future work should aim to test how sleep loss-related reductions in testosterone impact physical training and performance and how resilience-enhancing techniques, such as sleep extension, play a role.

As described, physical performance is linked to - and dependent on - testosterone. The findings presented here suggest if a Soldier attempts to exercise after a nocturnal training mission (or simply after not sleeping for other reasons), they may perform poorly, experience less muscle growth potential, or have poor recovery due to lower-than-normal testosterone levels. More critically, in a combat scenario, if Soldiers must perform a duty that requires sustained physical performance after having not slept, their performance and the mission could suffer. Ideally, Soldiers should obtain an adequate amount of sleep prior to executing missions requiring physical strength or stamina. If this is not feasible, Soldiers and military leaders should be aware that deficits

in physical performance could occur and should account for this during mission planning.

Limitations

Due to the nature of field work and to the difficulty of capturing biometric data during a specialized Ranger training mission, it is infeasible to account for all factors that may have impacted results. For instance, it has been proposed that high physical demand and caloric restriction associated with military training events (in combination with sleep loss) reduces testosterone [1]. Although the Rangers here may have gone several hours without refueling (food and water) while performing physical activities, this caloric restriction and physical activity was not as severe as the previous weeks- and months-long training missions [3,4]. Therefore, although it is certainly possible that those factors contributed to testosterone reductions observed here, we believe sleep deprivation - a strong physiological stressor - accounted for a large portion of the variance driving results. In order to better understand which aspects of training contributed to testosterone reduction, future studies should monitor additional factors (e.g., caloric intake, body mass index, skeletal muscle mass) and test additional, related compounds (e.g., cortisol and serotonin).

Further, future studies should include a control sample (e.g., a Ranger population that is sleeping during the nocturnal training mission timeframe) to more directly test the causal relationship between sleep loss and testosterone. In the current study, military trainings could not be modified to fit our research agenda, and we were therefore unable to include a well-rested control group. To compensate for this deficiency, we compared these data to a population that provided testosterone samples throughout the day and following a night of good quality sleep. By doing so, we demonstrated the extent to which testosterone was putatively impacted by sleep loss. However, there were limitations to this comparison, as testosterone was sampled during different times of day in each study (e.g., 0400 and 0500 vs. 0600), and the comparison population was not an elite Soldier population. Future studies should sample both the control and the experimental groups at the same time of day to rule out the influences of circadian variation on results.

Conclusions

The findings discussed suggest, preliminarily, that a single night of sleep deprivation during a U.S. Army special operations training mission is sufficient to significantly reduce testosterone. These data suggest elite athletes, both military and non-military, may perform poorly or have poor recovery following sleep loss. These data add to the growing body of literature showing that loss of sleep decreases testosterone, which may ultimately have a negative impact on physical training and performance in physically elite populations like the U.S. Army Rangers.

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Authors Contribution

All authors contributed substantially to the manuscript.

References

1. Linderman JK, O'Hara R, Ordway J (2020) Effect of Special Operations Training on Testosterone, Lean Body Mass, and Strength and the Potential for Therapeutic Testosterone Replacement: A Review of the Literature. *JSOM* 20: 94-100.
2. Mauras N, Hayes V, Welch S, Rini A, Helgeson K, et al. (1998) Testosterone deficiency in young men: marked alterations in whole body protein kinetics, strength, and adiposity. *J Clin Endocrinol Metab* 83: 1886-1892.
3. Nindl BC, Barnes BR, Alemany JA, Frykman PN, Shippee RL, et al. (2007) Physiological consequences of US Army Ranger training. *Med Sci Sports Exerc* 39: 1380-1387.
4. Morgan III CA, Wang S, Mason J, Southwick SM, Fox P, et al. (2000) Hormone profiles in humans experiencing military survival training. *Biol Psychiatry* 47: 891-901.
5. Hoedebecke K, Brink W (2014) Operational stressors on physical performance in special operators and countermeasures to improve performance: A review of the literature. *JSOM* 14: 84-85.
6. Kraemer WJ, Loebel CC, Volek JS, Ratamess NA, Newton RU, et al. (2001) The Effect of Heavy Resistance Exercise on Circadian Rhythm of Salivary Cortisol in Male Body Building Athletes. *Eur J Appl Physiol* 84: 13-18.
7. Luboshitzky R, Zabari Z, Shen-Orr Z, Herer P, Lavie P (2001) Disruption of the nocturnal testosterone rhythm by sleep fragmentation in normal men. *J Clin Endocrinol Metab* 86: 1134-1139.
8. Leproult R, Van Cauter E (2011) Effect of 1 week of sleep restriction on testosterone levels in young healthy men. *Jama* 305: 2173-2174.
9. DeCaro JA (2008) Methodological considerations in the use of salivary α -amylase as a stress marker in field research. *Am J Hum Biol* 20: 617-619.
10. Etikan I, Musa SA, Alkassim RS (2016) Comparison of convenience sampling and purposive sampling. *Am J Theor Appl Stat* 5: 1-4.
11. Bird SP (2013) Sleep, recovery, and athletic performance: a brief review and recommendations. *Strength Cond J* 35: 43-47.
12. Ritland BM, Simonelli G, Gentili RJ, Smith JC, He X, et al. (2019) Effects of sleep extension on cognitive/motor performance and motivation in military tactical athletes. *Sleep Med* 58: 48-55.
13. Rupp TL, Wesensten NJ, Bliese PD, Balkin TJ (2009) Banking sleep: Realization of benefits during subsequent sleep restriction and recovery. *Sleep* 32: 311-321.