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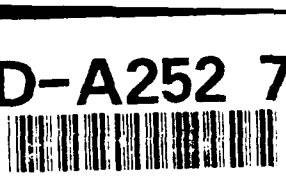

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THYROID HORMONE CHANGES DURING MILITARY FIELD OPERATIONS: EFFECTS OF COLD EXPOSURE IN THE ARCTIC

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Thyroid Hormone Changes During Military Field Operations: Effects of Cold Exposure in the Arctic

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Summary

This study examined the impact of prolonged physical activity in a cold environment on circulating thyroid hormone levels. A secondary focus of the study involved the role of nocturnal habitat upon the thyroidal responses to the physical activity and cold exposure. Military personnel exposed to 10 days of field-based operations in the arctic region of Norway were studied. Blood samples were collected before (day 1), and at days 5 and 10 of the operations. Levels of total T_4, free T_4, total T_3, free T_3, and thyroid binding globulin were assessed in all blood samples. Significant decreases ($p < 0.05$) in total thyroid hormone levels, and increases in free hormone fractions were found during the 10 days of operations in the arctic. However, no significant influence on thyroidal responses was observed due to the subjects' nocturnal habitat. The hormonal alterations noted are possibly brought about by the combined effects of physical activity and cold exposure acting synergistically to alter thyroid physiology (e.g., most likely the protein carrier binding affinity).

Introduction

Prolonged physical activity of a low to moderately intense nature over several days is known to introduce disruptions in the circulating levels of hormones. Aakvaag et al. (1) and Opstad & Aakvaag (16) demonstrated testosterone, total thyroxine (TT_4), total triiodothyronine (TT_3), prolactin, and growth hormone all decrease; while cortisol and the catecholamines increased with 5 days of semi-chronic physical activity (military training) in a neutral environment. Similar findings have been reported by other investigators (1,7,12,19). These changes occur for a variety of reasons; however, they primarily appear related to the hypocaloric states induced by a negative energy balance, stress reactivity of the endocrine system, and/or inappropriate thermoregulatory capability (2,16,22).

Many nations' military personnel periodically undergo sustained maneuvers involving prolonged physical activity to improve their military readiness. These "training exercises" involve exposure to an assortment of environmental extremes, with cold weather being a frequently encountered condition. With cold exposure there are many physiological adjustments to compensate for the thermal imbalance which may occur. One such adjustment is an elevation in the metabolic rate (to increase heat gain) via nonshivering thermogenesis (NST). A proposed mediator of NST is the circulating endocrine hormones, such as the adrenal catecholamines, the glucocorticoids, and/or the thyroids (24). Cold induced changes in thyroid hormones have been extensively investigated. Acute cold exposure is associated with increased thyroid stimulating hormone (TSH), TT_4, and TT_3 levels (4,8). Dissimilarly chronic cold exposure (of an extended duration), decreases the basal levels of the percentage free fractions of TT_4 and TT_3 (18). However, these acute and chronic hormonal changes are not universally reported (8,20). Little congenial information is available on the interacting effects of prolonged physical activity in a cold environment on the circulating levels of the thyroid hormones. Wilson (23) found after ~3 weeks in cold (12° to 8°C) surroundings (during which manual work and athletic training were performed), no significant change in thyroid parameters were noted in men. However, Solter et al. (21) reported factory laborers, after a work day involving cold exposure (8° to -40°C) and physical labor, had decreased TT_4, TT_3, and thyroid binding globulin (TBG) levels.

To examine the impact of physical activity in a cold environment on circulating thyroid hormone levels, we studied military personnel exposed to prolonged physical activity (10-day field operations) in the arctic polar region of Norway. A secondary focus of our study involved the role of nocturnal habitat upon the thyroidal responses to the physical activity and cold exposure.

Materials and Methods

Subjects. The subjects for this study were Norwegian infantry soldiers who were all normal, healthy, and in a euthyroid condition. Each subject signed an informed consent statement prior to participation. The subjects were arbitrarily divided into two nocturnal habitat groups. Group I (n=16) bivouacked in the field and lived in tents, while Group II (n=13) lived in barracks at a military garrison during the study. Throughout the study both groups underwent identical daily military field operations (noted below), were equipped/clothed identically, and consumed the same food rations. The subjects in both groups had completed equivalent levels of military service. The physical characteristics of the subjects at the onset of the study appear in Table 1.

Table 1.
Physical Characteristics of the Subjects at the Beginning of the Study.

Measure	Group	Mean	Standard Error
Age (yr)	I	20.3	0.2
	II	20.5	0.2
Height (cm)	I	181.9	1.3
	II	180.9	1.2
Weight (kg)			
Day 1	I	82.1	3.6
	II	79.9	3.7
Day 5	I	81.8	3.2
	II	78.6	3.3
Day 10	I	80.9	3.2
	II	78.5	3.4

Physical Activity. The field operations consisted of skiing and snow shoe marches, survival training, weapon training, and warfare maneuvers. The operations occurred in the Northern arctic region of Norway (approximately 340 km north of the arctic circle) during late winter. The training area was rolling hills, with elevations ranging from 300 to 1000 m above sea level. The weather consisted of daily intermittent sunshine and overcast, with occasional blowing snow and temperatures of + 5 to -20°C. Additionally, wind chill reductions of another 0 to -10°C occurred on a frequent daily basis. The typical daily exposure to the cold environment was between 10 and 12 h for both groups of subjects.

Blood Sampling & Analysis. Resting blood samples were taken on days 1, 5, and 10 of the study. The day 1 samples were taken with the subjects in a rest state (~48 h) and prior to the subjects commencing training or being exposed to the cold, therefore these were considered baseline assessments. The time of day for blood sampling was controlled and standardized. The samples were obtained via venipuncture and collected in an environmentally controlled field site (~20°C). Once collected, blood samples were kept cool (4°C) until centrifugation (2000 x g, 10 min). Separated sera was aliquoted and stored frozen at -50°C until biochemical analysis could be performed. This analysis consisted of TT_4, free thyroxine (fT_4), TT_3, free triiodothyronine (fT_3), and TBG. Additionally, the percentage of free fraction of T_4 ($\%fT_4$; fT_4/TT_4 x 100) and T_3 ($\%fT_3$; fT_3/TT_3 x 100) were calculated from the measures obtained. All hormonal assays were by radioimmunoassay procedures involving commercially available kits (thyroid hormones, DPC Inc., Los Angeles, CA, USA; TBG, Gammadab Clinical Assays, Los Angeles, CA, USA). In order to monitor the hydrational status of the subjects, hematocrit (%) and plasma protein concentration were measured in all blood samples. The hematocrit was determined via the micro-capillary tube method while the plasma protein was by refractometer. All biochemical analysis were performed in duplicate determinations.

At the same time as the blood samples were taken, each subject's body weight was assessed with a medical grade scale, and their oral temperature determined after an appropriate rest period via an automated system (Becton-Dickinson, Rutherford, NJ, USA).

Statistical Analysis. The data were analyzed with a between-within analysis of variance with repeated measures on the last factor. A Tukey HSD

post-hoc test was used to determine individual mean differences and the significance level was set at $p \leq 0.05$. All reported values are means $\pm$ SEM.

Results

The hormonal results for the individual groups are reported in Table 2. For TT_4, fT_4, and $\%fT_4$ no differences ($p > 0.05$) were observed between Group I or Group II at any point in the study. However, significant effects ($p < 0.001$) were seen across the 10 days of the study for TT_4, fT_4, and $\%fT_4$. The day 10 TT_4 level was significantly lower than the day 1 and day 5 values (see combined group means [CGM] results, Figure 1), but no difference was observed between the day 1 and 5 values. The fT_4 (Figure 2) was significantly elevated at day 10 from the day 5 value, but not the day 1 value. However, the day 1 and day 5 fT_4 values did not differ from one another. For $\%fT_4$, no differences were noted between the day 1 and 5 values, but the $\%fT_4$ at day 10 was significantly greater than both the day 1 and 5 values (see Figure 3).

INSERT FIGURE 1-3 ABOUT HERE

Table 2. Thyroid Hormonal Changes of the Subjects (X ± SEM).

Measure	Group	Day 1	Day 5	Day 10
TT_4	I	5.80	5.60	4.28
		±0.18	±0.20	±0.10
	II	6.76	6.36	4.92
		±0.34	±0.26	±0.26
fT_4	I	1.11	1.01	1.18
		±0.04	±0.03	±0.04
	II	1.20	1.16	1.28
		±0.04	±0.03	±0.05
$\%fT_4$	I	19.42	18.46	27.90
		±0.66	±0.50	±1.10
	II	18.20	18.71	26.70
		±0.38	±0.50	±0.76
TT_3	I	196.28	161.48	144.16
		±12.28	±3.91	±4.18
	II	199.78	164.53	157.70
		±9.42	±6.24	±5.86
fT_3	I	3.72	4.04	3.71
		±0.12	±0.09	±0.10
	II	3.73	4.14	3.60
		±0.10	±0.13	±0.10
$\%fT_3$	I	2.08	2.54	2.61
		±0.13	±0.08	±0.08
	II	1.95	2.57	2.35
		±0.08	±0.09	±0.09

The group responses for TT_3, fT_3, and $\%fT_3$ were similar to those of the T_4 measures (see Table 2). That is, no differences ($p > 0.05$) were observed between Groups I and II at any point in the study, but significant ($P < 0.01$) time

effects occurred for all the measures (see the CGM results in Figures 4-6). The TT_3 at days 5 and 10 were significantly lower than the day 1 value, but not different from each other (Figure 4). The fT_3 level (Figure 5) at day 5 was significantly greater than the day 1 and 10 values. But the day 1 and 10 fT_3 values were not found to differ from one another. The $\%fT_3$ at day 5 and 10 were significantly greater than the day 1 value, yet they were not different from one another (see Figure 6).

INSERT FIGURE 4-6 ABOUT HERE

The individual group TBG results were as follows for day 1, 5, and 10, respectively: Group I = 25.9 $\pm$ 0.8, 26.1 $\pm$ 0.6, 25.8 $\pm$ 0.7 ug/ml; Group II = 26.2 $\pm$ 0.6, 26.3 $\pm$ 0.7, 24.4 $\pm$ 0.7 ug/ml. No significant differences ($p > 0.05$) existed between the groups or occurred across the 10-day time period for this measure.

Table 3. Select Physiological Measures of the Subjects (X $\pm$ SEM).

Measure	Group	Day 1	Day 5	Day 10
Hematocrit (%)	I	44.71	44.04	44.87
		$\pm$0.51	$\pm$0.44	$\pm$0.33
	II	44.67	44.61	45.46
		$\pm$0.58	$\pm$0.76	$\pm$0.39
	CGM	44.70	44.30	45.13
		$\pm$0.37	$\pm$0.41	$\pm$0.25
Plasma Protein (g/dl)	I	7.84	8.10	8.17
		$\pm$0.10	$\pm$0.08	$\pm$0.10
	II	7.82	7.94	8.13
		$\pm$0.07	$\pm$0.09	$\pm$0.08
	CGM	7.83	8.03	8.15
		$\pm$0.06	$\pm$0.06	$\pm$0.06
Oral Temp (°C)	I	36.7	36.5	36.4
		$\pm$0.1	$\pm$0.2	$\pm$0.1
	II	36.7	36.4	36.5
		$\pm$0.2	$\pm$0.2	$\pm$0.2
	CGM	36.7	36.4	36.4
		$\pm$0.1	$\pm$0.1	$\pm$0.1

Hematocrit was comparable between the groups throughout the study, and there was a slight increase by the 10th day in both groups (see Table 3), but this change was not significant ($p > 0.05$). The plasma protein concentrations followed a similar trend as the hematocrit. But, a statistically significant change in plasma protein for the CGM results was observed over time (Table 3). The day 5 and day 10 values for the measure were greater ($p < 0.01$) than the day 1 value, and the day 10 was greater than that at day 5. Oral temperatures of the groups were similar throughout the 10 days, and no significant changes across time were observed (Table 3). Nonetheless, there was a decline in the oral temperatures of the subjects at days 5 and 10, and this change nearly reached statistical significance ($p = 0.06$, see CGM in Table 3).

The body weights of both Group I and II were comparable across the course of the study. The weight did decline progressively (see CGM, $p < 0.02$) in the subjects, and was significantly reduced from day 1 levels by day 5; and declined slightly more (significantly) from day 5 to 10 (see Table 3).

Discussion

The purpose of our study was twofold; 1) to examine the impact of military operations involving prolonged physical activity in a cold arctic environment on circulating thyroid hormone levels, and 2) to determine what role nocturnal habitat played upon the thyroidal responses to physical activity in a cold environment. As to the first, our data demonstrated that considerable, significant changes occur in thyroid hormones with 10 days of military operations in an arctic setting. However, with regard to the second, no significant influence on thyroidal responses was observed due to the habitat the subjects were assigned to.

In examining the hormonal data, it is important to look at the possible role of exercise-induced, or cold-related hemoconcentrating effects on blood based measures (11). This was indirectly assessed in our subjects on the basis of the hematocrit and plasma protein responses. The results suggest that while a small degree of hemoconcentration may have occurred, for the most part, our subjects were well hydrated. This finding argues against a substantial impact of hemoconcentration on the hormonal responses we are reporting.

We found significantly lower TT_4 and TT_3 by the end of the 10 days of cold exposure. These results agree with other studies that have looked at just prolonged physical activity, or just sustained cold exposure only (1,5,10,18).

Yet, they are at odds with the exercise or cold exposure studies that have examined acute responses in humans (9,14,15). In those studies, however, the subjects typically exercised for less than 1 h, and the cold exposure was of a similar short-term duration. Thus, the applicability of these acute response studies to the present study seems ill-suited.

The physiological mechanism by which the total hormone levels became reduced is uncertain. Both animal and human based studies would suggest these findings represent a cold adaptation response in our subjects (3,18). This adaptation is possibly a central regulated event involving reduced thyrotropin (TSH) and thyrotropin-releasing hormone (TRH) release (6). Unfortunately, methodological restraints did not allow for us to measure TSH or TRH in the present study, and thus possibly shed some light on the issue. An additional consideration is that inadequate nutrition played a role in producing the reduction in the TT_4 and TT_3 levels. That is, the physical activity of the subjects was at a substantial level throughout each day of the study. Previously published data from this study indicate the daily average heart rates for the subjects were approximately 100 bpm. Therefore, it is possible that the subjects were unable to compensate for the high energy expenditure with an adequate caloric intake, thus they developed a negative energy balance. This was in spite of them being allowed to eat ad libitum. The reduction of circulating TT_4 and TT_3 levels is a classic response to malnourishment or starvation-like states in humans (13). The reductions in the body weight of the subjects would support that they were in a negative energy balance, especially in lieu of the factor that minimal dehydration seemed to occur. That is, only small amounts of the weight loss appear fluid related based upon the hematocrit and plasma protein changes. However, it is realized that these hydration measures are not ideal indicators of the overall fluid status of humans. Why TT_4 and TT_3 reductions are associated with inappropriate nutrition are unclear (13). Hyposecretions of TSH and/or TRH have been suggested, but currently evidence is too contradictory to be confident in this conclusion.

The fT_4 and fT_3 data are less clear since significant increases in the free fractions occurred, but at incongruous points in time. That is, the fT_4 was significantly elevated only by day 10, but the fT_3 became elevated at day 5 and then returned to previous levels. The interpretation of these free fraction changes is aided when the percentage measures are studied. These data reveal the

$\%fT_4$ and $\%fT_3$ were significantly elevated at days 10, and 5-10, respectively. Such a change in the free fractions of the hormones in a state of reduced total hormone levels is consistent with decreased binding of the hormones to protein carriers (21). Free fractions of the thyroid hormones can be altered by the total amount of protein carriers, but we demonstrated no significant changes occurred in the TBG concentrations. Although TBG is not the only protein carrier for thyroid hormones, it does account for ~70-75% of the circulating bound hormones and has the greatest hormone affinity. It is uncertain how binding affinities of the carrier proteins for the thyroids may become altered. Galbo (9) reports that physical activity alone will decrease protein affinity for hormones via the transient temperature shifts in the blood during exercise. Fregly (8) suggests that the affinity changes, allowing for enhance free fractions, may be an adaptation response to the cold to improve those physiological functions influenced by the thyroid hormones. In the present study, the changes brought about seem most likely due to a synergistic effect of the physical activity and the cold upon the subjects. It could be argued that our subjects were possibly not truly cold. That is, they lived in the micro-environment provided by the military cold weather uniforms. However, investigator observation revealed that they were repetitively exposed to cold conditions, especially their face and hands, on a frequent basis. Furthermore, while by no means conclusive, the trend towards a lowered oral temperature does give some support to the thought that they were cold.

With respect to the secondary purpose of this study (nocturnal habitat influence), we were most astonished by the lack of an effect. It had been hypothesized that an enhanced environmental stress would be placed upon the field subjects (Group I). However, clearly and consistently there were no statistically significant effects on the thyroid hormones due to this factor. The explanation for the lack of an effect may be due to the great care the field subjects took in preparing and maintaining their tent quarters. We observed that these living spaces, while small and somewhat cramped, were thermally very comfortable. Furthermore, the subjects were extremely diligent in maintaining the thermal status of their quarters constantly. Therefore, the subjects may have eliminated the potential for added cold exposure at night because of their conscientious behavior.

In conclusion, we found significant decreases in total thyroid hormone levels, and increases in free hormone fractions during 10 days of military operations in an arctic setting. These alterations are most probably brought about by the combined effects of physical activity and cold exposure acting synergistically to alter thyroid physiology; most likely the protein carrier binding affinity. However, no significant influence on thyroidal responses were observed due to the nocturnal habitat that the subjects lived in.

The direct application of these results remains to be determined, but the findings do suggest that military operations in cold environments can induce changes in the thyroid status of soldiers. Ultimately, thyroid hormone changes have the potential to have both negative and positive effects on the functional metabolism of these soldiers, which undoubtedly could effect their military readiness.

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Figure Legends

Figure 1. This figure depicts the total thyroxine (TT_4 changes during the 10 days of the military operations). Significant changes ($p < 0.01$) from day 1 values are denoted with an *. All values are means $\pm$ SEM.

Figure 2. This figure depicts the free thyroxine (fT_4 changes during the 10 days of the military operations). Significant changes ($p < 0.01$) from day 1 values are denoted with an *. All values are means $\pm$ SEM.

Figure 3. This figure depicts the percentage free fraction of thyroxine ($\%fT_4$ changes during the 10 days of the military operations). Significant changes ($p < 0.01$) from day 1 values are denoted with an *. All values are means $\pm$ SEM.

Figure 4. This figure depicts the total triiodothyronine (TT_3 changes during the 10 days of the military operations). Significant changes ($p < 0.01$) from day 1 values are denoted with an *. All values are means $\pm$ SEM.

Figure 5. This figure depicts the free triiodothyronine (fT_3 changes during the 10 days of the military operations). Significant changes ($p < 0.01$) from day 1 values are denoted with an *. All values are means $\pm$ SEM.

Figure 6. This figure depicts the percentage free fraction of triiodothyronine ($\%fT_3$ changes during the 10 days of the military operations). Significant changes ($p < 0.01$) from day 1 values are denoted with an *. All values are means $\pm$ SEM.

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13. ABSTRACT *(Maximum 200 words)*

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