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We exposed 18 lean, lightly-clothed women, who were users of oral contraceptives, to cold air to create a stressful situation for evaluating the effects of supplemental tyrosine upon subjective reactions and mental performance. We studied these women, during days 15-26 of their pseudomenstrual cycles, when concentrations of plasma tyrosine would be least and supplemental tyrosine might be most beneficial. Participants were exposed twice to 7°C air for 90 min. Tyrosine or maltodextrin (placebo) was incorporated into a specially-developed nutrient bar; tyrosine supplementation (9.3 g) was double blinded. A third condition, 23°C and placebo, was a baseline for temperature. We evaluated participants' symptomatology, moods, and cognitive performances with laptop computers; determined plasma levels of tyrosine, epinephrine, norepinephrine, and cortisol; and recorded skin and body temperatures and heart rate. Our nutrient bar methodology was a feasible way to administer the supplemental nutrient, tyrosine, double-blinded. Tyrosine improved ratings of Clear Thinking after 86.5 min of cold exposure. Eight subjective states were affected by cold; only Clear Thinking was improved by tyrosine. Supplemental tyrosine did not improve any measures of mental performance. Substantial cold stress was documented; however, it remains to be determined if the stressful conditions were adequate for a definitive evaluation of supplemental tyrosine.

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Louis E. Bauhut 20 Jan 97

PI - Signature Date

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Final Report for Work Effort Number D1950110

TESTING THE EFFICACY OF ADMINISTERING TYROSINE TO REDUCE ENVIRONMENTAL STRESS (COLD) IN WOMEN

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INTRODUCTION

Several animal and human experiments demonstrated that when supplemental tyrosine is administered before a stressful challenge, animals and humans tolerate the stressor better than controls that are untreated.[1,4,14,15,17,21,26] A recent Danish study showed that women who take combination oral contraceptives have lower concentrations of plasma tyrosine during days 16 to 25 of their menstrual cycle than women who do not use oral contraceptives.[20] Since users of oral contraceptives have lower levels of circulating chemical substrate (tyrosine) than what is normal during this phase of their menstrual cycle, these women with depressed levels of plasma tyrosine may be more vulnerable to varied stressors. We chose to investigate whether administration of supplemental tyrosine would reduce adverse changes in subjective reactions (symptoms and mood states) and mental performance since increased chemical substrate or levels of plasma tyrosine seem adaptive in dealing with varied stressors, especially if there is a deficiency or increased turnover of plasma tyrosine.[14,15,17,28]

Our prior studies with tyrosine investigated the combined stressors of exposure to high altitude and 16°C. Tyrosine reduced the adverse effects of these combined stressors upon symptomatology, mood states, and mental performance, but we did not learn if tyrosine is effective for just cold alone.[1,2,17] Since soldiers may be exposed to many situations that involve cold stress (low ambient temperatures, wind chill, increased cooling caused by wet clothing from perspiration or precipitation), we wanted to determine if tyrosine alleviates some of the adverse effects of cold stress upon symptomatology, mood states, and mental performance. This multidisciplinary

investigation (Appendix A) was to evaluate if supplemental tyrosine reduces the adverse effects of cold exposure (e.g., symptoms, adverse moods, performance impairments) in women using oral contraceptives. Potential beneficial effects of supplemental tyrosine were evaluated with measures of cognitive performance, subjective reactions, thermoregulation, and catecholamines. Support and funding for this project were provided by the Defense Women's Health Research Program.

EXPERIMENTAL METHOD

Eighteen military and civilian women, 18-34 years of age, using combination oral contraceptives, and with $\leq$ 27% body fat (~30-40th percentiles in young military women)[9,10] participated in this study. We used dual energy x-ray absorptiometry (DEXA)[18] to select participants with leaner body compositions so they would experience more uniform and marked responses to cold. All testing was scheduled and completed during days 15-26 of a participant's pseudomenstrual cycle. Cognitive performance and subjective reactions (Appendix B) were measured with laptop computers;[2,3,27] thermoregulatory indices, with automated equipment[5]. Core temperature was measured with a thermistor probe inserted 10 cm into the rectum. Skin temperature was measured with thermocouples attached at five sites (thigh, chest, triceps, forearm, and leg). We used a modification of the weighting formula by Newburg and Spealman[13] so that mean skin temperature was calculated as follows:

= .34 chest + .075 triceps + .075 forearm + .33 thigh + .18 leg

Tyrosine or placebo was administered double-blinded (cross-over, counterbalanced design) in a specially-developed nutrient bar (Appendix C) during two exposures to cold, i.e., 7°C for 90 min. The nutrient bar, containing 9.3 g of tyrosine or 9.3 g of maltodextrin (placebo), was ingested 35-40 min before each of the two cold exposures. Participants were also tested in 23°C for 90 min (thermoneutral baseline) so that the effects of cold could be documented. Before the 23°C exposure, participants ingested a nutrient bar (placebo). Participants fasted from 2200 h each evening before exposure to an environmental and nutrient condition. Participants were lightly-clothed (Champion™ sports bra and performance bottom) during each of the three test sessions. The daily schedule for all project activities and the schedule for psychological testing are shown in Appendices D and E; respectively. Each participant was issued a Champion™ sport uniform for the study. Each participant who completed the study was paid $600 for initial medical evaluation and practice with the assessment tasks(three sessions)and the three experimental sessions.

An effect was statistically significant if the likelihood of its occurrence by chance was $p \leq 0.05$. Main effects were evaluated with repeated-measures ANOVAs; the significance of specific data comparisons (given a significant main effect) was

determined with the Tukey method or paired-t tests. In some of the statistical analyses, potential outliers were identified by statistical programs. Although elimination of such outliers would have often improved statistical significance, we analyzed and reported the original data.

RESULTS

Physical Characteristics Data. Forty eight women volunteered for this study. Thirty women were excluded because a few had preexisting medical conditions that were contraindicated for this study or their measurements with the DEXA exceeded our body fat criterion ($\leq$ 27%). The participants' physical characteristics of percent body fat, age, height, and weight for the women that we studied and those that we excluded are shown in Appendix F.

Tyrosine Assay (Plasma). Fig. 1 shows that administering nutrient bars with supplemental tyrosine increased plasma tyrosine. The bars resulted in a 10-fold increase in plasma tyrosine (2 1/4 h after ingestion) compared to an average, initial value of 31.82 μmol/l before the nutrient bars were administered. This increase in plasma tyrosine was significantly different than values associated with administration of placebos during the 7°C and 23°C exposures. Participants indicated that the nutrient bars were palatable and enjoyable to eat.

Thermal Data. Rectal temperatures, skin temperatures, and peripheral heat loss are shown in Fig. 2-3 for the various combinations of 7°C and 23°C and formulations of the nutrient bar (supplemental tyrosine or placebo) studied. The control values were measured after 70 min of rest and equilibration in ambient conditions, just before participants entered the chamber to begin the environmental exposure (Appendix D). During the first 60-70 min of the cold exposure, average core temperature increased approximately 0.2°C and then decreased so that after 70-90 min average core temperature was slightly less than initial control values (Fig. 2). The core temperature data for the 7°C conditions (tyrosine and placebo) was not different. In the 23°C condition, core temperature was maintained relatively constant over the 90-min exposure.

Core temperatures decreased little from control values and such responses were not decreased for a sustained period. Analysis of individual core temperatures after 70 min of cold exposure indicated, 50% of the participants showed no decreases from control values (in some cases there were increases in body temperature). Another 33% of the participants experienced body temperatures that were $\leq$ 0.20°C colder than control values. After 90 min of cold exposure, 2 min after all psychological testing was completed, 17% showed no decrease (or they showed an increase) in core temperature, 56% had core temperatures $\leq$ 0.30°C than control values, 22% had decreases $\leq$ 0.60°C, and 6% had decreases of $<$ 0.90°C.

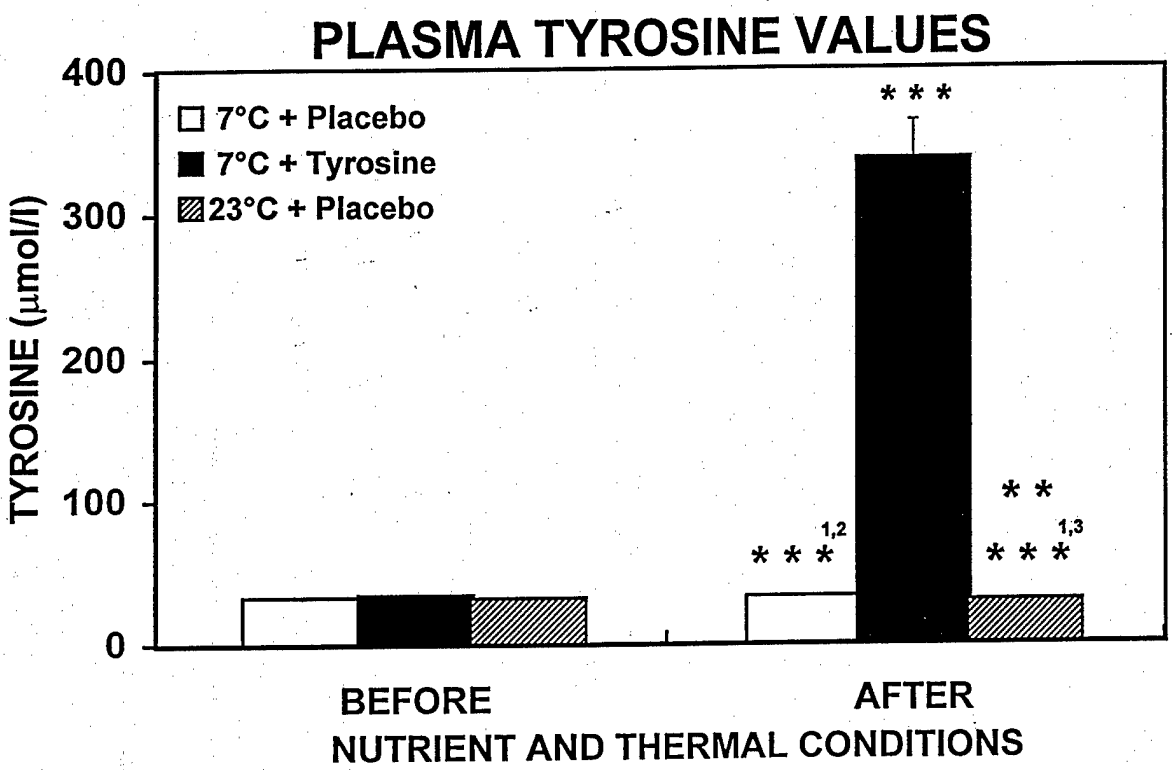

Fig. 1--Plasma tyrosine values 'Before' and 'After' nutrient and environmental manipulations. A bracket above a bar is 1 standard error of the mean (SEM). SEMs, plotted for the other bars, are not visible. Asterisks indicate statistical significance: *, **, and *** equal $p \leq 0.05$, 0.01, and 0.001; respectively. Asterisks (without superscripts) indicate significance between 'Before' and 'After' values for a specific condition (e.g., 7°C + Tyrosine). Asterisks (with superscripts) indicate significance between bar graphs specified in that time sample, e.g., In the 'After' sample, plasma tyrosine for 7°C + Tyrosine was significantly different than for 7°C + Placebo.

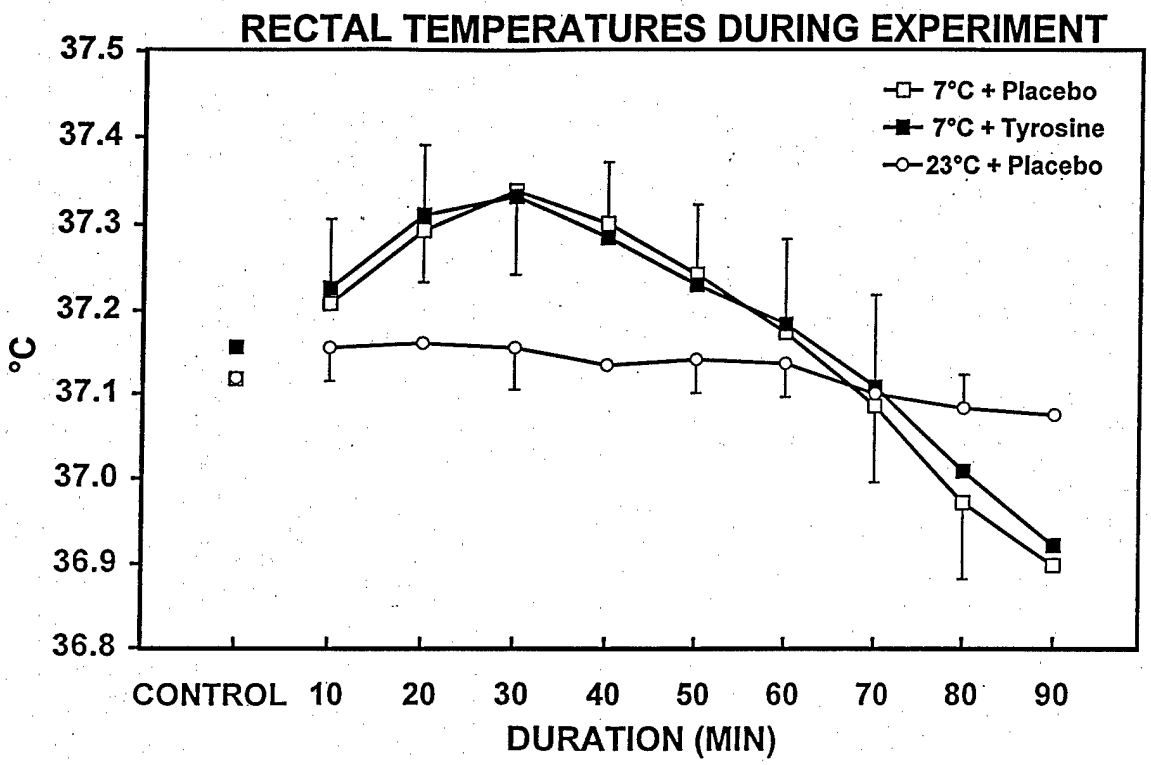

Fig. 2--Rectal temperatures (average) during each 90-min session evaluating different nutrient and environmental conditions. The control values shown for each condition were measured after 70 min of equilibration in ambient conditions. After determination of control values, participants entered the chamber and experienced a nutrient and environmental condition. The bracket above (or below) each data symbol shows 1 SEM.

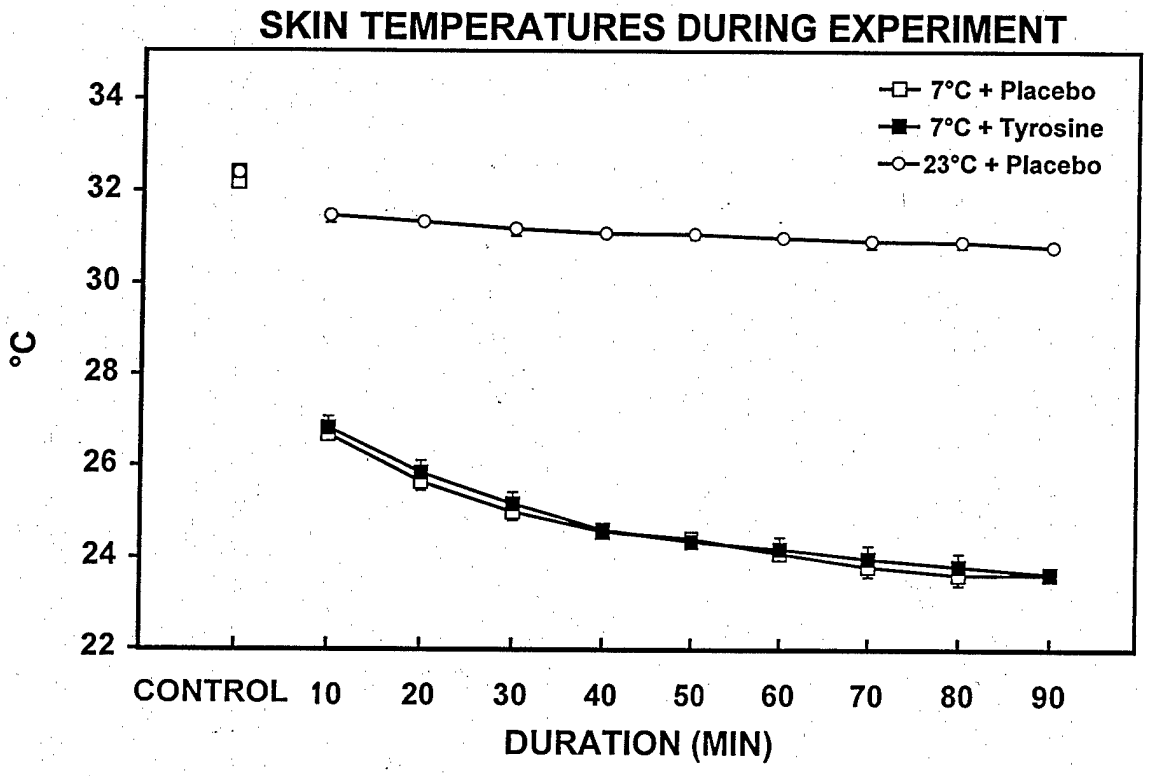

Fig. 3--Mean skin temperatures for each 90-min session in which different nutrient and environmental conditions were evaluated. Control values for each condition were measured in ambient conditions; then, participants entered the chamber and experienced a nutrient and environmental condition. The bracket above (or below) each data symbol is 1 SEM; not all are visible at this scale.

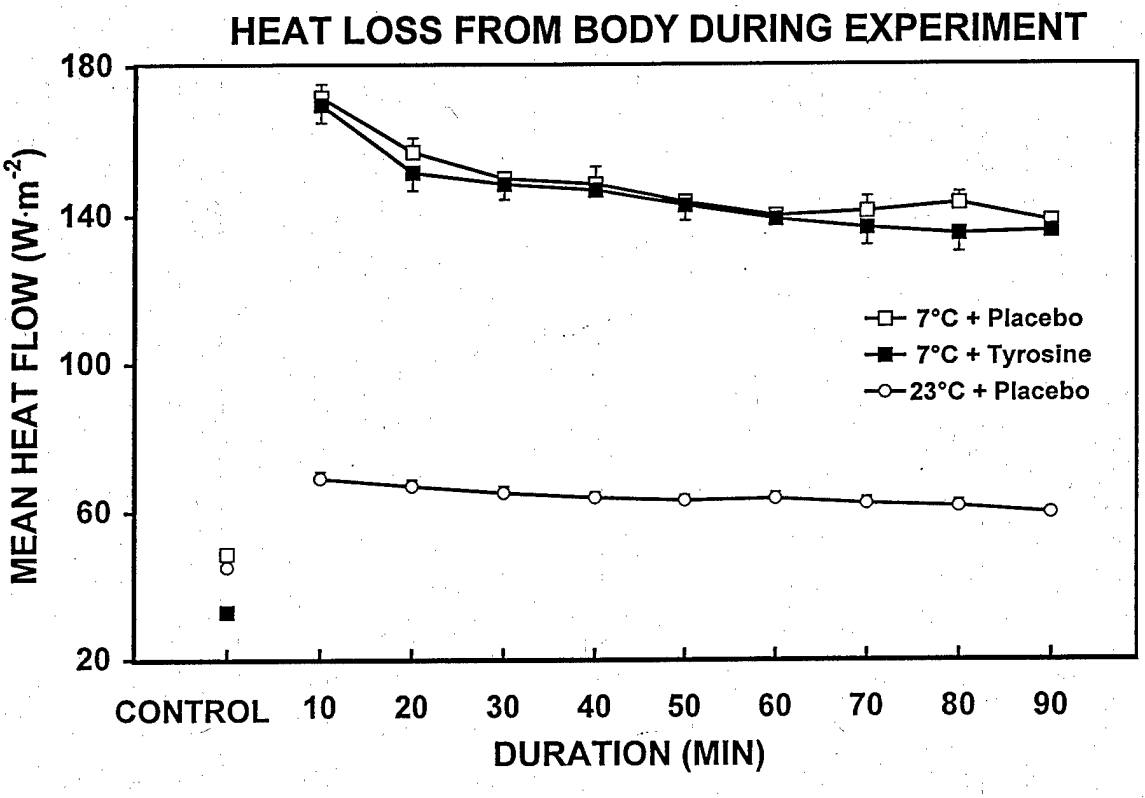

Fig. 4--Heat loss (average) from our 18 participants during each 90-min session investigating different nutrient and environmental conditions. After a control value for each condition was measured in ambient conditions, participants entered the chamber and experienced a nutrient and environmental condition. Not all brackets for the SEM are visible at this scale.

Skin temperatures decreased ~5°C within the first 10 min of the cold exposures (Fig. 3). After 70-90 min they decreased ~3°C more. The 23°C condition resulted in only a 1°C decrease below control values for skin temperatures. Initially, during the cold exposure, peripheral heat loss was about two and one half times greater than that for 23°C; after 20 min of the cold exposure, heat loss was twice as great (Fig. 4) A participant's percentage of body fat and her decrease in core temperature after 90 min of the environmental exposure were correlated ($r = -0.52$, $p \leq 0.05$). No participants ever reached the lower medical safety limit for core temperature (35°C), and no participants ever chose to end their involvement in the study during a cold or thermoneutral exposure.

Behavioral Data. Mental Arithmetic[3] was evaluated at 17, 69, and 77 min (midpoint of each assessment interval) during each environmental exposure. Inspection of these data (number of problems correct per session) suggested that the participants' performances improved substantially with each session. A statistical test (ANOVA) conducted on the performance data for Mental Arithmetic yielded a significant Participant by Day interaction ($p \leq 0.01$). Performance on the Matching-To-Sample task, assessed at 33 min, yielded one statistically significant dependent variable ($p \leq 0.05$) which indicated that this performance was degraded by cold.

We also assessed the effects of supplemental tyrosine upon subjective reactions as measured in symptoms and moods. Fig. 5 shows a significant interaction between the nutrient condition (supplemental tyrosine or placebo) and the duration of the cold exposure as manifested by the Clear Thinking factor of the Clyde Mood Scale.[6] After administration of tyrosine and 86.5 min of cold exposure, ratings of Clear Thinking were significantly greater than they were in participants administered placebo. This happens because ratings of Clear Thinking were sustained with time in volunteers pretreated with tyrosine; volunteers given placebo exhibited less Clear Thinking after 86.5 min than they had initially during the cold exposure. This treatment effect of tyrosine reduced the effects of cold exposure.

Exposure to cold for 84.5 min resulted in increased ratings of coldness compared to those after only 6.5 min (Fig. 6). Ratings of coldness during the two cold exposures (tyrosine and placebo conditions) were not different. The two 7°C conditions were rated colder than the 23°C condition. Fig. 7 shows the mood states of Friendliness, Sleepiness, and Dizziness measured by the Clyde Mood Scale after 86.5 min of environmental exposure.[6] Cold exposure caused participants to be significantly less friendly, less sleepy, and more dizzy than when they were exposed to 23°C. Although cold exposure caused changes in these mood states, ratings for these mood states were not different when tyrosine or placebo was administered before exposure to cold. Likewise, Fig. 8 shows three mood states after 82.5 min measured by the Profile of Mood States (POMS)[19]. Exposure to cold produced greater Tension, Confusion, and Total Mood Disorder; however, scores were

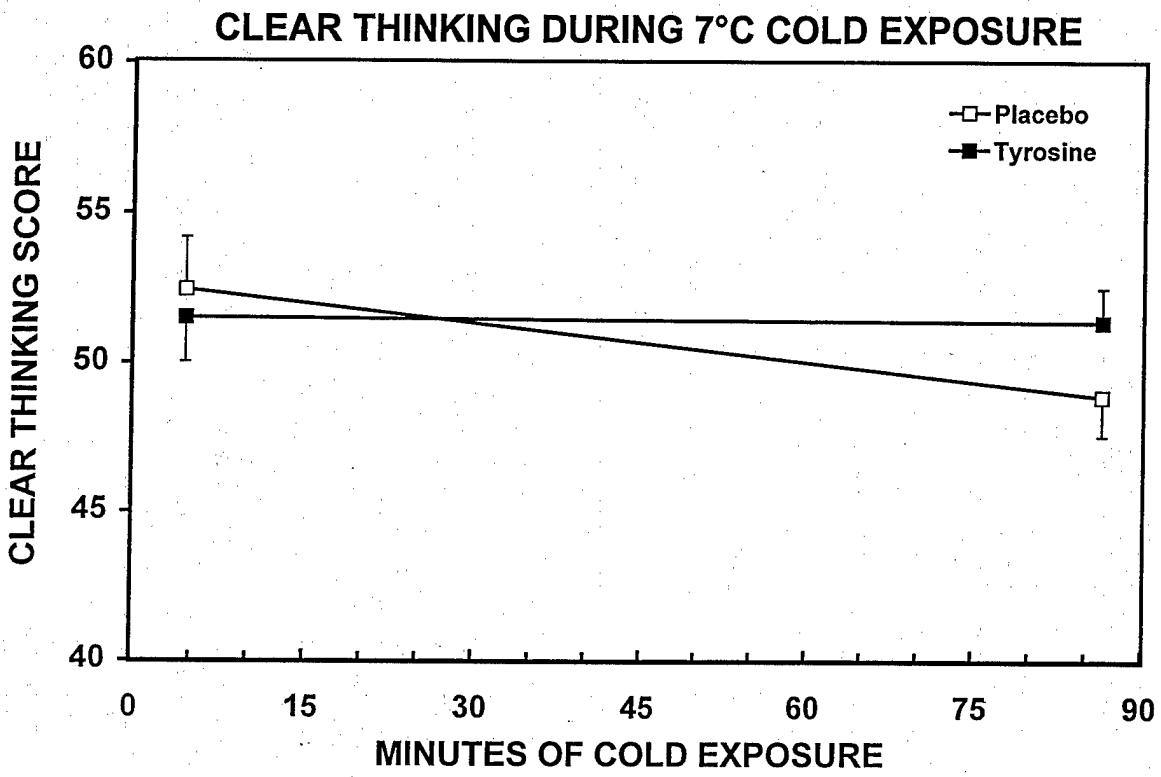

Fig. 5--Average ratings of Clear Thinking during 4.5 min and 86.5 min of two 7°C cold exposures. The solid-square symbol indicates when participants were pretreated with tyrosine before a cold exposure; the open-square symbol indicates when they were pretreated with placebo.

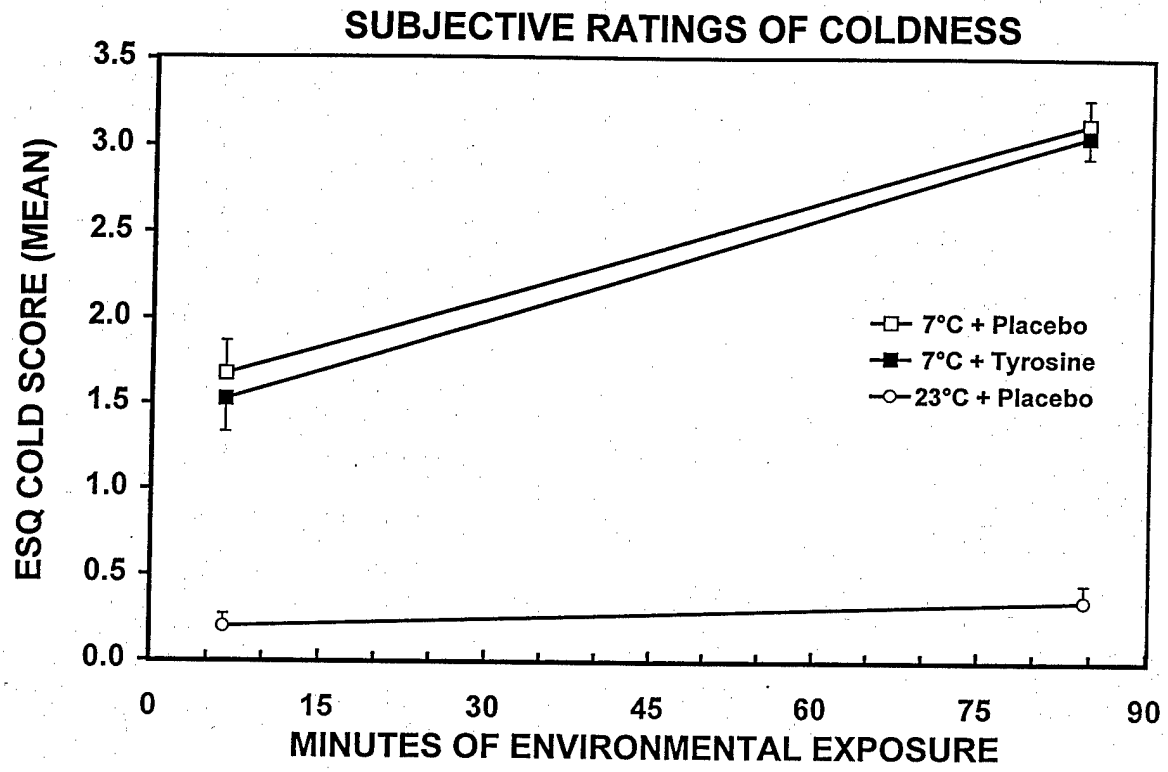

Fig. 6--Average ratings of Coldness for three nutrient and environmental conditions. Ratings were determined after participants were in the chamber 6.5 min and 84.5 min. A bracket above (or below) a data symbol shows 1 SEM.

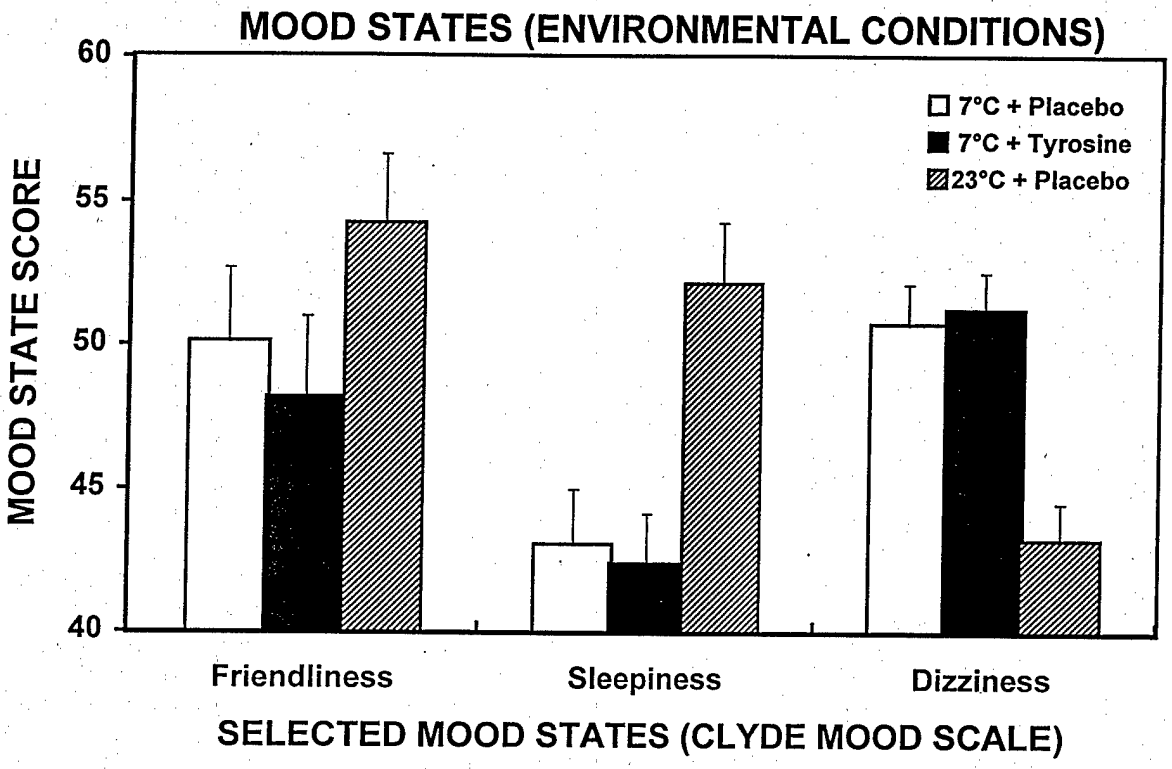

Fig. 7--Average ratings of Friendliness, Sleepiness, and Dizziness by 18 participants during the 90-min environmental and nutrient conditions. These measurements were determined after 86.5 min of the 90-min session. A bracket above each bar shows 1 SEM.

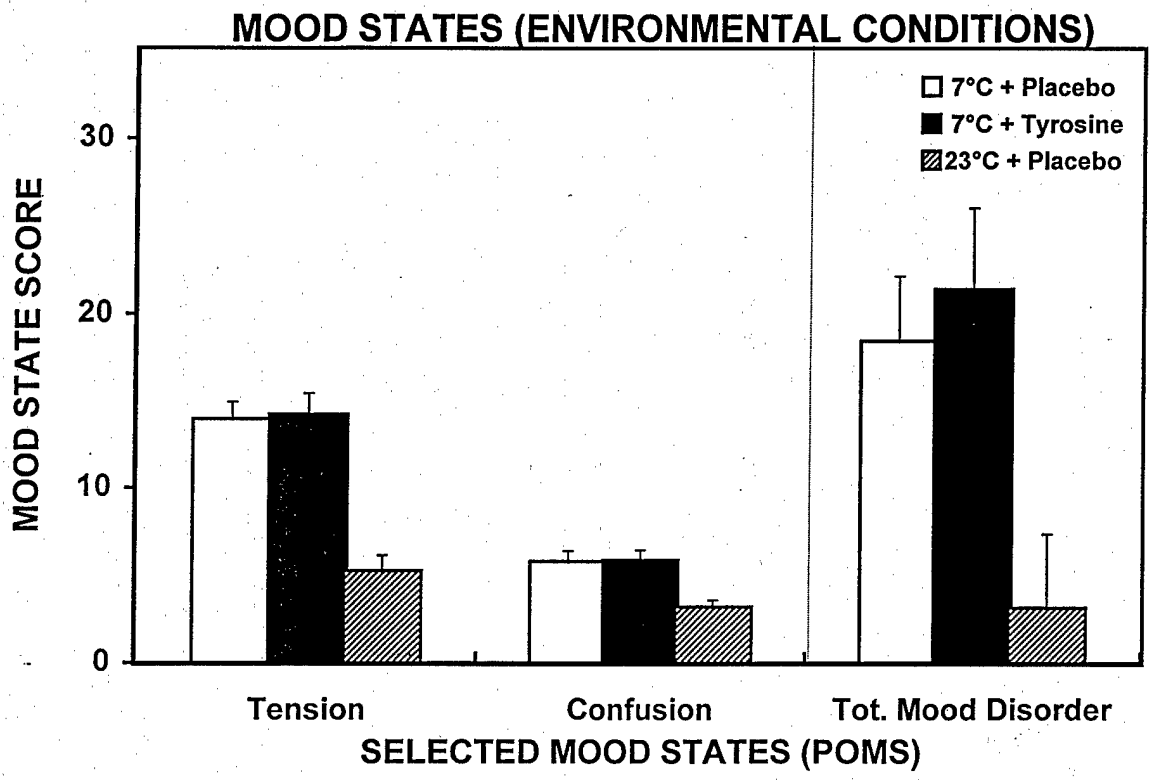

Fig. 8--Average ratings of Tension, Confusion, and Total Mood Disorder after 82.5 min of each environmental and nutrient condition. The bracket above each bar shows 1 SEM.

not different during cold exposure when participants were pretreated with tyrosine or placebo.

Above, we only reported psychological measures, evaluated from 69 to 86.5 min (and when these measures were administered earlier in the environmental session), because there were minimal changes in core temperature before 70 min of cold exposure. We also reported the data for the Match-To-Sample task which occurred earlier in session (21-45 min) because of its similarity to a Navy study with men that demonstrated tyrosine reduced some of the adverse effects of cold exposure upon memory.[26]

Neurochemical Data (Plasma). In Fig. 9-11 and Fig. 1, the first blood sample, 'before,' was drawn in a quiet area adjacent to the environmental chamber just before the nutrient bar was administered, ~50 min before the beginning of the environmental exposure. The second blood sample, 'after,' was drawn in the environmental chamber immediately after 90 min of environmental exposure (Appendix D). The 'after' values are the most relevant comparisons for this study, however, 'before' values are also arrayed. Testing of each participant was performed across as many as 12-days; the 'before' averages and SEMs in these figures suggest remarkable consistency of values for participants from session to session.

Epinephrine levels in plasma were increased after 90 min of exposure to 7°C (supplemental tyrosine or placebo) than exposure to 23°C (Fig. 9). Values for tyrosine and cold exposure versus placebo and cold exposure did not differ significantly. Norepinephrine increased significantly after environmental exposure for all conditions of temperature and nutrient bar (tyrosine and placebo) as shown in Fig. 10. Exposure to cold increased norepinephrine far greater than exposure to the warm condition. Supplemental tyrosine and cold exposure resulted in a smaller, statistically significant, decrease in norepinephrine than did the combination of cold exposure and placebo.

In Fig. 11, cortisol concentrations were greatest before each environmental exposure (7°C or 23°C) and associated administration of the nutrient bar. Each 'before' measure was significantly greater than the corresponding 'after' measure. Although cortisol has a marked periodicity in the morning[11], the magnitude of our measured changes suggests that anticipation of the environmental sessions was also a factor (we did not tell participants what the environmental condition would be each session; often participants did not know).

Drawing blood samples from indwelling catheters in women participants after 90 min of cold exposure proved difficult. After the cold exposures, some values of epinephrine and norepinephrine were probably influenced by the increased effort and trauma associated with drawing blood samples from women in the cold and the use of a tourniquet. Also, there probably was greater variability in the descriptive statistics of some plasma assays after cold exposure. Sometimes, we could not draw a blood sample from a participant in the cold so the number of participants included in some group statistics changed.

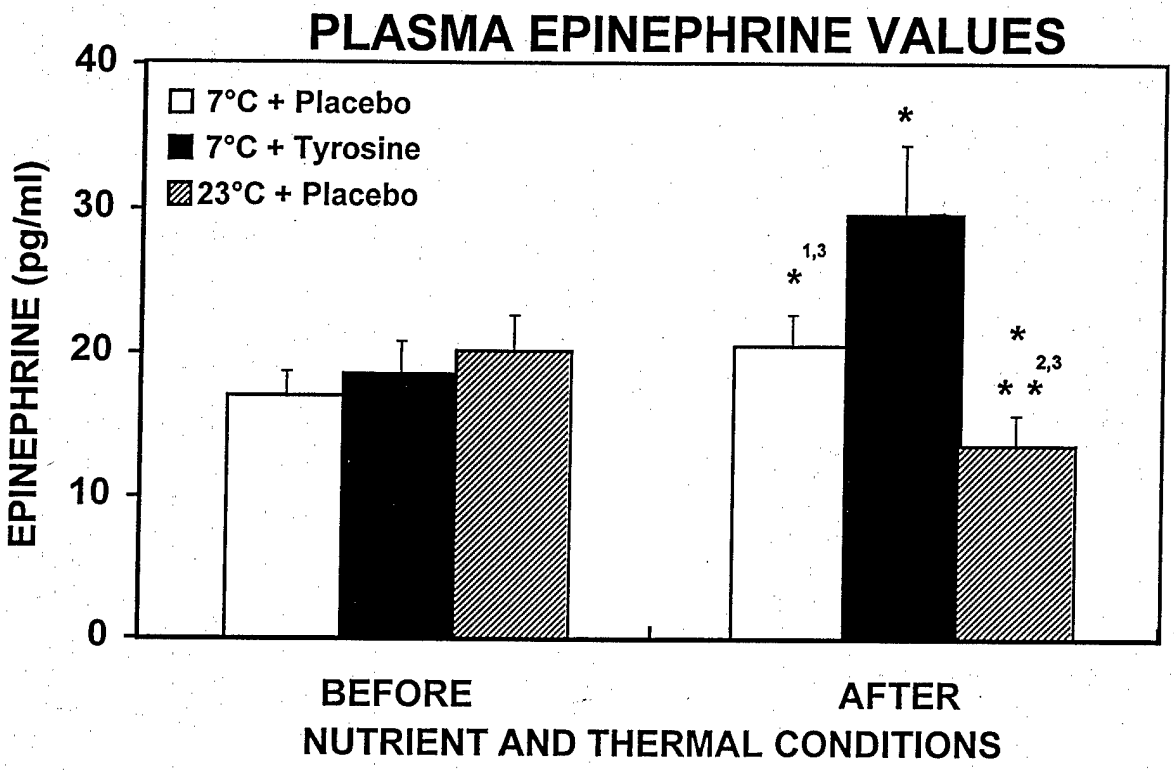

Fig. 9--Plasma epinephrine values sampled 'Before' and 'After' three nutrient and environmental manipulations. The bracket above each bar is 1 SEM. The number of asterisks indicates the level of statistical significance. Asterisks (without superscript numbers) show the statistical significance between 'Before' and 'After' values for a specific environmental and nutrient manipulation, e.g., 7°C + Tyrosine. Asterisks (with superscripts) indicate a significant difference between the bar graphs specified in that time sample, e.g., plasma epinephrine for 7°C + Tyrosine was significantly greater than for 23°C + Placebo in the 'After' sample.

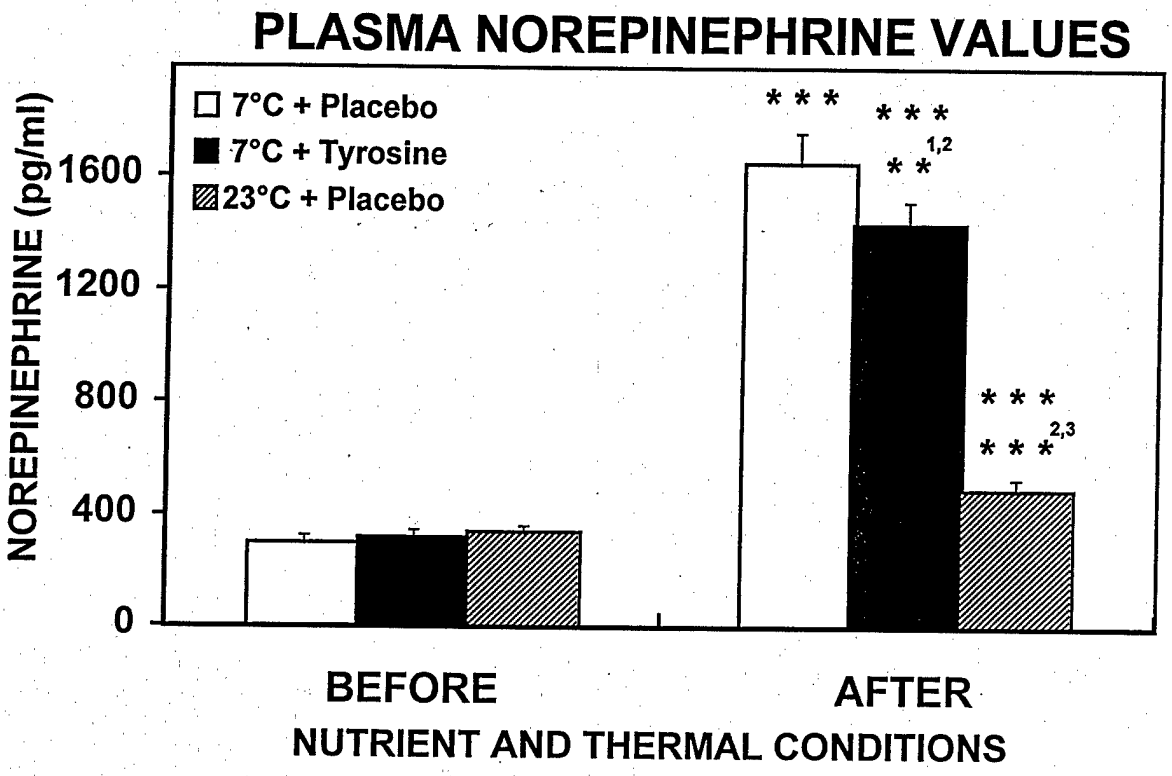

Fig. 10-- Plasma norepinephrine values sampled 'Before' and 'After' various nutrient and environmental manipulations. Asterisks (without superscript numbers) indicate statistical significance between 'Before' and 'After' values for a specific environmental and nutrient manipulation, e.g., 7°C + Tyrosine. Asterisks (with superscript numbers) indicate significant differences between the bar graphs specified in that time sample.

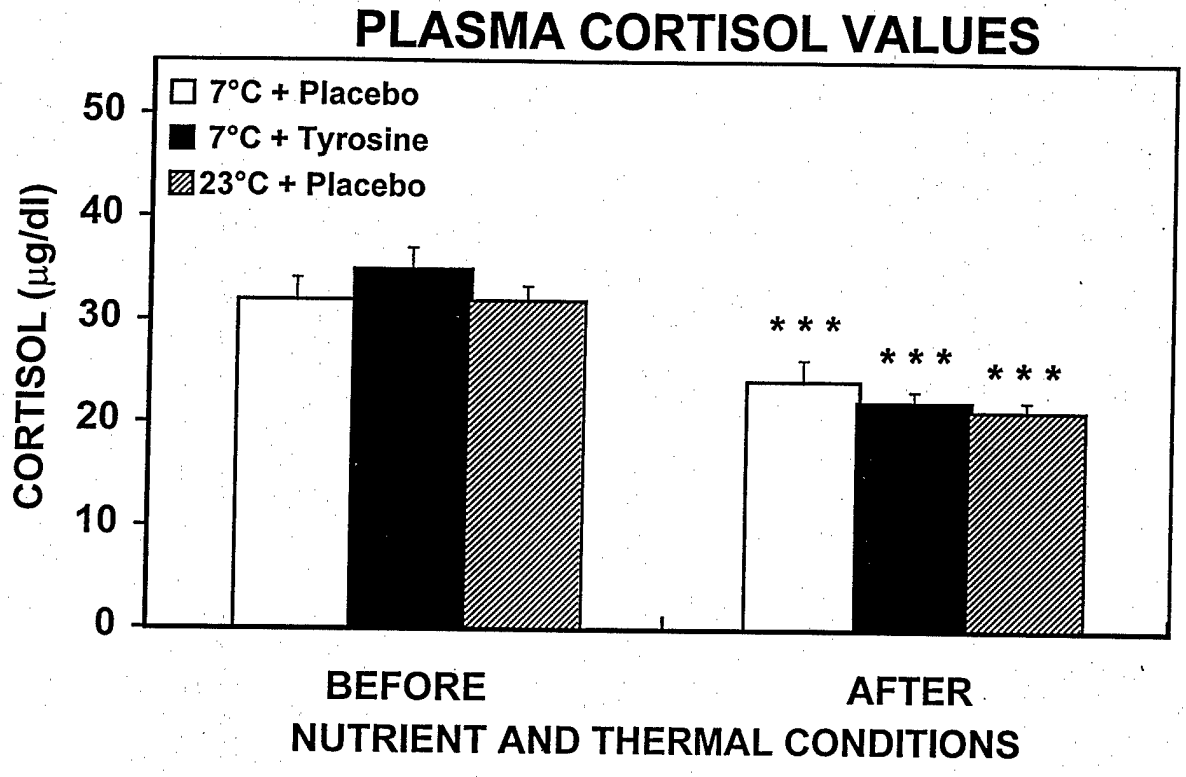

Fig. 11-- Plasma cortisol values sampled 'Before' and 'After' various nutrient and environmental manipulations. Asterisks (without superscript numbers) indicate statistical significance between 'Before' and 'After' values; asterisks (with superscript numbers) indicate significant differences between the bar graphs specified within a time sample.

DISCUSSION

This study was to determine if administration of supplemental tyrosine reduced some of the adverse effects of cold exposure as evaluated with our indices of objective performance and subjective reactions. After 77 min of cold exposure, performance on our Mental Arithmetic task was not different in volunteers pretreated with tyrosine as compared to placebo. Several subjective measures yielded significant effects attributable to exposure to cold; however, Clear Thinking was the only subjective measure that yielded a significant tyrosine effect. Furthermore, there were no instances where the effects of placebo were significantly better than supplemental tyrosine for any of our performance or subjective measures.

Clear Thinking was improved by tyrosine. Although all 48 adjectives in the Clyde Mood Scale contribute slightly to this statistical factor, the four adjectives that contribute most are efficient, alert, clear thinking, and able to concentrate. This suggests that supplemental tyrosine improves "states of being" regarded as desirable and conducive for mental activity. A comparable improvement in Clear Thinking was also documented in a high altitude study at 4,300 m.[24] The improvement (magnitude of change) in Clear Thinking after 28-42 h at high altitude was the same magnitude (~4 units) as that observed in this study.[24] This improvement in Clear Thinking with time at high altitude was statistically significant ($p \leq 0.01$).

We used a Matching-To-Sample task in this study, but did not replicate the Navy's finding of a tyrosine effect during cold exposure. There were many differences between our studies. We used women with a greater percentage of body fat than would be expected for their Navy male counterparts (body fat not reported). Our environmental exposure was 7°C; theirs was 4°C. We tested on the Match-To-Sample task after 21-45 min of exposure; they tested after 30-60 min. Lastly, our participants had less practice before the experiment than did the Navy personnel. During both practice and experimental sessions our participants performed other performance tasks and measures to assess subjective reactions. Experience and proficiency with multiple tasks probably created interference and hindered practice for our participants on the Match-To-Sample task. Such factors, especially gender and practice, probably accounted for the our inability to replicate the Navy's finding.

This study was our first effort at including supplemental tyrosine in a formulation, much like a high energy bar (carbohydrates, calories, fat) that could be used for administering supplemental nutrients to soldiers in the field. The increased levels of plasma tyrosine observed in this study compare favorably with those from an earlier study where we used gelatin capsules to administer supplemental tyrosine.[17] The nutrient bar formulation was highly regarded by this study's participants and staff. Participants (and scientific personnel) were not able to distinguish the nutrient bars containing tyrosine from those without tyrosine under the conditions of administration in this study. Many of our participants were

calorie-conscious dieters; all enjoyed the bar and did not find it too filling. This formulation of the nutrient bar appears a convenient and natural way of administering supplemental nutrients in a manner that is highly acceptable to research participants and scientific staff.

This multidisciplinary study involved several scientific and technical personnel; however, it was successfully executed and varied data were collected. Plasma samples for assays of tyrosine and the catecholamines were obtained although drawing blood samples after personnel were exposed to the cold proved to be difficult. The psychological testing and collection of the thermal measures were done entirely by computer. Food scientists from the Natick Research, Development, and Engineering Center and a commercial food products company combined their expertise to produce a nutrient bar to administer supplemental tyrosine or the placebo condition for this study. We also screened participants for their percentage of body fat and only accepted participants with $\leq$ 27% body fat. The substantial empirical association between a participant's percentage of body fat and her drop in body temperature after 90 min of cold exposure suggests that using percentage of body fat as a selection criterion for participants was a useful strategy for creating more homogeneous temperature responses and larger decreases in body temperature during cold exposure. Lastly, each participant displayed great motivation for the study and the psychological testing.

There were indications that our participants experienced substantial cold stress during the 90-min exposure to cold. Thermal (heat loss and skin temperatures) responses to the cold were very rapid. Within the first 10 min, there was increased heat loss through the skin and more than a 5°C decrease in skin temperature. Vasoconstriction of peripheral vessels, pooling of the blood inward towards the body's core, and shivering increased body temperature within the first 30 min of the exposure. Concentrations of epinephrine and norepinephrine increased dramatically after 90 min of cold exposure. Behaviorally, a number of our subjective measures detected the effects of cold (e.g., Coldness, Clear Thinking, Friendliness, Tension, Confusion).

Although it is impossible to determine from these data, it is possible that the conditions in the present study were not sustained long enough to produce marked cumulative stress (e.g., as reflected in reduced core temperatures) and adverse psychological effects that could be ameliorated by supplemental tyrosine. Although eight of our subjective measures yielded statistically significant effects of cold, only one yielded a significant treatment effect. Our prior work suggests that subjective reactions (symptoms and mood states) are usually affected by stress before measures of cognitive performance.[25] Selection of participants with $\leq$ 27% body fat provided greater thermal responses and less variability to cold; however, after 60-70 min our participants' core temperatures were minimally decreased by the conditions of this study. No participants withdrew from the study during evaluation of any environmental and nutrient conditions; no participants were ever withdrawn

because they exceeded the medical safety limit for reduced body temperatures. We have numerous instances in our study where the outcome for tyrosine during the cold exposures is in the correct direction (a dependent variable with supplemental tyrosine is better than that variable with placebo); however, such trends are usually not statistically significant (e.g., mood and symptom data). However, it remains to be determined whether tyrosine might have had a more demonstrable effect if conditions were colder or sustained longer.

Most studies in animals[4,14,15,17,21] that demonstrated beneficial effects of supplemental tyrosine used highly aversive paradigms like unavoidable shock to the tail, severe restraint, and 'cold soaking' of rats to lower their body temperatures to 30°C. Such studies suggest that substantial (if not life-threatening and highly emotion-provoking) stress may be necessary to produce adverse effects that can be reversed with supplemental tyrosine. It is likely that the qualitative properties of the stressor and the intensity of the stressor influence behavioral responses to the stressor. Experimental studies with humans in the cold with administration of supplemental tyrosine are few; our own prior studies involved exposure of sedentary participants to 4700 m simulated altitude and 16°C for up to 7 h--conditions which towards the end of the session were uncomfortable but not cold.[1,2,17] A Navy study of men lightly clothed and exposed to 4°C demonstrated supplemental tyrosine reduced errors on a memory task assessed after 30-60 min of cold exposure.[26] Lastly, this study demonstrated improvements in women's ratings of Clear Thinking after 86.5 min of exposure to 7°C.

In future efforts, careful consideration and deliberation should be given to a paradigm to create appropriate conditions. Perhaps stressful physical manipulations (heavy physical work and sleep deprivation) before treatment with supplemental tyrosine in a multi-stressor paradigm, or use of military populations whose training and mission demands subject them to much stress (Rangers, Special Forces), or psychological manipulations (simulated military boards or training presentations), and elements from other paradigms can be used to design a future study that will lead to a robust evaluation of the ability of supplemental tyrosine to reduce stress in soldiers and foster generalization to military groups.

CONCLUSIONS

1) Supplemental tyrosine improved performance on the mood state of Clear Thinking after 86.5 min of cold exposure.

2) The nutrient bar, used in this study, was a feasible way to administer supplemental nutrients in double-blind studies. It was highly palatable to the participants and differences between the supplemental tyrosine and the placebo formulations were not detectable during administration of the nutrient.

3) This study successfully incorporated several multidisciplinary methodologies and documented cognitive performances and thermal, neuroendocrine, and subjective indices.

4) Several manifestations of substantial cold stress were documented in this study. It remains to be determined whether supplemental tyrosine would have more pronounced beneficial effects under more severe conditions of cold stress.

5) In future studies, careful consideration should be given to creating an appropriate paradigm to evaluate tyrosine and generalize the results. Other paradigms such as an experimental laboratory investigation with demanding physical manipulations before introduction of cold stress or the use of military groups that are normally exposed to excessive stress during their training and execution of their mission should be carefully considered.

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APPENDIX A

ACKNOWLEDGEMENTS

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A multidisciplinary study requires the support, commitment, and dedication of many critical personnel. First, we thank our test participants who were committed, cooperative, and professional. They are one of finest group of test participants the first author has ever worked with. The project brought together professional from diverse disciplines. We have learned from each other, our different perspectives, and expertises.

The Principal Investigator is especially indebted to Ms. Jennifer Collins and SGT Sabrina Carson. Both contributed heavily to the study preparation and execution phases of the study. Their skills, enthusiasm, and professionalism did much to ensure success of many of the administrative and logistical aspects of this project. Ms. Donna J. Merullo was responsible for many of the analyses of the data; her exceptional care with data and statistical skills were great assets. SPC E. DeJesus is also recognized for the illustrations in the report; they are testimony to his newly-developed Excel™ skills and personal growth during his initial months at USARIEM.

The Principal Investigator also recognizes the contributions of Dr. Ed Ross, Dr. Irwin Taub, Mr. Ray Spring, and Mr. Robert F. Wallace for their insights and suggestions on possible alternative analyses of the data.

We are also indebted to CPT Verne L. Backus, CPT Jonathan Canete, and the Staff at the US Army Natick Occupational Health Clinic for their medical evaluations and lab testing of all women who volunteered for this study. The Medical Staff's willingness to accommodate the schedules of potential participants greatly increased our ability to recruit them. Lastly, there were many people who worked behind the scenes ordering supplies, ensuring that participants were paid, preparing blood samples for analysis, and providing other services.

APPENDIX B

DESCRIPTIONS OF PSYCHOLOGICAL TESTS USED TO MEASURE SYMPTOMS, MOODS, AND COGNITIVE PERFORMANCE

Assessment of Symptoms, Mood, and Cognitive Performance

We evaluated all participants with multiple, dependent measures of symptomatology, mood, and cognitive performance described below. We administered these measures with lap-top computers with colored displays. Before test sessions 1-3, we provided extensive practice on the various measures of symptoms, mood, and cognitive performance (e.g., 2-9 administrations depending upon the measure). This minimized practice effects.

During each test session, each measure was administered one or more times and sequenced so that all psychological testing could be completed in 90 min (Appendix D). The order of the tests and questionnaires each test session was always fixed (Appendix E). Alternate, but equivalent, forms of the performance tests were used for all repeated testing. Participants were sedentary and were tested almost continuously during each test session.

USARIEM Visual Vigilance Test

This test of visual vigilance was designed to resemble military tasks requiring sustained scanning of the visual environment for infrequent, difficult to detect stimuli such as those during sentry duty.[8] The task required the participant to detect a faint dot that appeared somewhere randomly on the screen for two seconds. On the average, presentation of the dot occurred once each min. When the stimulus was detected, the participant pressed the space bar on the keyboard as quickly as possible. The computer recorded whether or not a response occurred (e.g., stimulus was detected) and the response time for detections. Responses made before (or after) stimulus occurrence were recorded as false positives. A session lasted 13 min.

Four-Choice Visual Reaction Time Test

Ability to react rapidly is a key factor underlying a number of critical military duties. Response latencies are susceptible to stress imposed by the external environment and other situations.[7] Participants were presented with a series of visual stimuli at one of four different spatial locations on the computer screen to assess visual reaction time.[16,17] The participant's task was to indicate the correct spatial location of each stimulus by striking one of four adjacent keys on the computer keyboard. The measurements recorded included: the response latency for each trial, premature errors (responding before the presentation of the stimulus), errors of omission (response latency >1 sec) and errors of commission (hitting the wrong key). The test required 5 min.

Delayed Match-to-Sample Test

Working memory is critical for many military and civilian duties. This test assessed both short term and spatial memory and was sensitive to tyrosine and cold exposure.[26] This test is a subtest of the Walter Reed Performance Assessment Battery; a sample pattern of 36 red and green grid squares was presented on the computer display. The research participants studied this pattern and then pressed a key on the keyboard. The sample pattern disappeared; then, the screen was blank for 4 seconds ("delay"). Next, two patterns were presented and the participant selected the pattern that matched the previous sample pattern. The delayed match-to-sample test consisted of several trials and required 24 min to complete.

Mental Arithmetic Task

Participants performed the Mental Arithmetic Task.[3] This task required rapid and accurate mental calculations, decision making, and an action that reflected the participant's decision. Addition problems, and plausible sums, were displayed on the screen of a lap-top computer positioned directly in front of each participant. The computer generated a display of 36 vertical addition problems. Each problem had three, two-digit numbers and a plausible sum. The participant decided if each sum was "correct" or "incorrect." A participant indicated her decision by pushing the "F" or "J" key on the lap-top. The participant completed some practice problems; then, after 7 min the task ended and the number of problems completed during the session was displayed as feedback for the participant. This task was responsive to the effects of altitude and exhibited sensitivity that was superior to a paper-and-pencil test and another automated mental arithmetic test.[3]

The Automated Addition Task presented incorrect sums for 50% of the problems. Participants mentally calculated each problem's sum and decided if it differed from the sum given by the computer. The algorithm for generating the problems was designed so that deviations of ± 1 occur in the 100, 10, or 1-place digit of the sums on 2.5, 45.0, or 2.5% of all problems; respectively. Participants can not change their response after they press a key on the keyboard.

Stanford Sleepiness Scale (SSS) Questionnaire

The SSS is a computer-administered scale of seven descriptive statements of a person's state of sleepiness.[12] Each participant indicated the statement that most closely described her state of sleepiness at that time; this required less than 1 min.

Clyde Mood Scale

The Clyde Mood Scale consisted of 48 adjectives, e.g. "good-natured", "troubled", "lonely", "impulsive", which were rated on a discrete anchor point scale ("not at all", "a little", "quite a bit", and "extremely"). For each adjective, a participant indicated her rating by keying a number that corresponded to the discrete anchor point that she chose for that adjective. This mood scale required 3 min to complete and resulted in six different factors: Friendliness, Aggressiveness, Clear Thinking, Sleepiness, Unhappiness, and Dizziness.[6]

Environmental Symptoms Questionnaire (ESQ): Cold Factor

Nine items (15,19, 28, 31, 34-37, and 61) selected from the 67-item Environmental Symptoms Questionnaire were assessed to determine each participant's rating of cold stress.[22,23] These nine items yielded a statistical factor of 'cold stress' on the complete questionnaire.[22,23] The items are statements like "My hands are shaking or trembling," "I feel weak," "I feel chilly," and "I'm shivering." Participants rated each statement using a 6-point scale with discrete anchor points which varied from "NOT AT ALL" to "EXTREME." This questionnaire required 1 min.

Profile of Mood States Questionnaire

The questionnaire is an inventory of subjective mood states.[19] Each participant used a lap-top computer to rate a series of 65 mood-related adjectives on a five point scale, using the response set of "How are you feeling right now?" The POMS required 3 min to complete. Previous research has shown that the adjectives factor into six mood subscales (Tension, Depression, Anger, Vigor, Fatigue, and Confusion).

APPENDIX C

DESCRIPTION OF NUTRIENT BAR:

MATRIX FOR ADMINISTRATION OF SUPPLEMENTAL TYROSINE

SPECIALLY-DEVELOPED HIGH ENERGY BARS FOR ADMINISTERING TYROSINE (OR PLACEBO) TO RESEARCH PARTICIPANTS

Personnel from NRDEC and USARIEM are evaluating specially formulated food bars; this is a developmental effort in the Performance Enhancement Ration Components Project. The bars were developed through a cooperative research and development agreement initiated by NRDEC personnel and a commercial company that is extensively involved in performance research and the marketing of energy bars to enhance performance. To date, berry, banana, and chocolate matrices have been developed. These matrices have been carefully formulated and balanced for user acceptability with simple or complex carbohydrates, protein, and lipid composition. These matrices are designed for incorporating performance enhancing agents such as tyrosine, caffeine, glutamine, and simple or complex carbohydrates for validation by testing performance with physiological and psychological indices.

The chocolate matrix was used in this study. Its ingredients were glucose, corn syrup, fructose, chocolate, maltodextrin, crisp rice (rice, sugar, malt, salt), partially hydrogenated soybean oil, concentrated fruit (dates, plums, figs), glycerol, cocoa powder processed with alkali. The quantities and types of nutrients in the tyrosine and placebo formulations are shown below.

HIGH ENERGY BAR: CHOCOLATE MATRIX

Serving Size: 65 g Total Calories: 285 Calories from Fat: 52

	TYROSINE BAR		PLACEBO BAR	
CONSTITUENTS	**grams**	**%(weight)**	**grams**	**%(weight)**
TOTAL FAT	5.8	8.7	5.8	8.7
Saturated Fat	2.3	3.4	2.3	3.4
CHOLESTEROL	0	0	0	0
SODIUM	0.112	0.17	0.112	0.17
TOTAL CARBOHYDRATE	50	75	59.3	88.5
Sugars	27	40	27	40
Maltodextrin	0	0	9.3	13.9
DIETARY FIBER	1.8	2.7	1.8	2.7
TOTAL PROTEIN	9.3	13.9	0	0
Tyrosine	9.3	13.9	0	0

This effort was the first of several possible strategies for administering nutrients, drugs, or medications in a desirable and palatable foodstuff. The varied matrices hide the taste of substances to be evaluated in natural foodstuffs so that they can be administered 'blinded'. This methodology is also advantageous for administering nutrients in greater quantities than would be possible in their natural form as a food. Although it was possible to make up the nutrient bars with different amounts of

tyrosine so that the dose (e.g., mg/kg) would be similar for participants of differing body weights, this was judged unnecessary. We anticipated over 80% of the participants that we would test will be within ± one standard deviation of the mean (heavier women will be less likely to meet the body composition criterion). We predicted that most of our participants will ingest 138-178 mg/kg. Furthermore, we are not aware of any studies of tyrosine that have shown subjective and performance effects that are dose-related. In fact, our own studies did not demonstrate dose-responsive effects for doses of 100 or 150 mg/kg[1,2] and 80 or 170 mg/kg.[17]

In the present study, we felt that the potential variability that may be added when a fixed quantity of tyrosine was administered in a high energy bar was not critical nor did it compromise the scientific objectives of this study. This study with a supplemental nutrient administered in a food bar may prove critical in developing a methodology for conveniently administering other nutrients and beneficial substances within a palatable foodstuff.

APPENDIX D

DAILY SCHEDULE OF EVENTS FOR THE EXPERIMENT

Table I-- Schedule of daily events during the experiment.

TYPICAL DAILY SCHEDULE FOR TYROSINE STUDY

CLOCK TIME	ELAPSED TIME	EVENT
07:15	0	Arrive
07:15-07:35	0-19	Change into clothing, void bladder, insert internal thermal sensor
07:35-08:25	20-50	Instrument and Insert Venous Catheter

NOTE: Although the immediately prior event usually required 20-30 min, 50 min were scheduled each day. The time line below lists the most typical outcome--Participants were all ready after 20-30 min so they began resting at 0800 h. (If more time was required to instrument and catheterize the participants, they began resting at 0825 h and each subsequent event was 25 min later than shown).

08:00-08:20	0-20	Rest 20 min--Collect baseline physiological measurements
08:20-08:30	20-30	Draw First blood sample
08:30-08:35	30-35	Ingest nutrient bar and water
08:35-09:10	35-70	Rest 35 min--Allow uptake tyrosine
09:10-09:15	70-75	Enter chamber; start thermal measurements
09:15-10:45	75-165	Psychol. testing in environmental conditions
10:45-10:55	165-175	Draw second blood sample
10:55-11:15	175-195	Exit chamber, ingest hot liquids and oatmeal, remove instrumentation
11:15-11:30	195-210	Change clothes; leave Institute

APPENDIX E

DAILY SCHEDULE FOR PSYCHOLOGICAL TESTING

Table II--Schedule of psychological testing during the environmental exposure.

TEST EVENT	DURATION (min)	ELAPSED (min) TEST SESSION	MIDPOINT (min) TEST EVENT
TRANSITION	3	0-3	1.5
CLYDE_MOOD SCALE	3	3-6	4.5
ESQ_(COLD SCALE)	1	6-7	6.5
STANFORD SLEEPINESS SCALE	1	7-8	7.5
FOUR-CHOICE REACTION TIME	5	8-13	10.5
MENTAL ARITHMETIC	8	13-21	17
MATCH-TO-SAMPLE	24	21-45	33
BREAK	7	45-52	48.5
VISUAL VIGILANCE TEST	13	52-65	58.5
MENTAL ARITHMETIC	8	65-73	69
MENTAL ARITHMETIC	8	73-81	77
MOOD SCALE (POMS)	3	81-84	82.5
ESQ_(COLD SCALE)	1	84-85	84.5
CLYDE_MOOD SCALE	3	85-88	86.5
ENVIRON. SESSION COMPLETED	--	90	--

APPENDIX F

PHYSICAL CHARACTERISTICS OF PARTICIPANTS WHO WERE INCLUDED (OR EXCLUDED) IN THE STUDY

Table III--Physical characteristics of participants who were included or excluded from the investigation.

VARIABLE	PARTICIPANTS TESTED (N=18)	EXCLUDED (N=30)
Age		
Mean ± S.D.	23.67 ± 4.84	23.23 ± 4.78
Median	21.50	21.00
Height (cm)		
Mean ± S.D.	164.25 ± 8.71	162.22 ± 9.04
Median	165.10	161.29
Weight (kg)		
Mean ± S.D.	54.61 ± 6.19	60.33 ± 7.18
Median	56.47	58.97
% Body Fat (DEX A)		
Mean ± S.D.	23.74 ± 3.04	30.89 ± 3.99
Median	24.60	30.50

APPENDIX G

NOTIFICATION OF PUBLICATIONS

PUBLICATIONS

Published reports from this study will be forwarded to the DWHRP as they become available.

MEETING ABSTRACTS

Published reports from this study will be forwarded to the DWHRP as they become available.

CONTRACTUAL PERSONNEL

Computer Programmer (Student)

Nurse (IV Catheter Specialization)

Research Assistant (Student)

Statistical Assistant (GeoCenter's Employee)

16 Volunteers (Civilians who completed the study)

32 Volunteers (Civilians who completed 1-4 sessions)

ADDENDUM: COMMENTS TO ISSUES OF THE REVIEWER AND COR

WORK EFFORT: D1950110

PROJECT: Testing the Efficacy of Administering Tyrosine for Reducing Environmental Stress (Cold) in Women

PRINCIPAL INVESTIGATOR: Louis E. Banderet, Ph.D.

ASSOCIATE INVESTIGATORS: H.R. Lieberman, Ph.D.; A.J. Young, Ph.D., CPT J.W. Castellani, Ph.D., COL Valerie Rice, Ph.D.

CONTRACTING ORGANIZATION: U.S. Army Research Institute of Environmental Medicine, Natick, MA 01760-5007

REPORT DATE: December 1996

1. Was an adequate sample size used for this project? Do the dependent measures have adequate power to detect cold and treatment effects for these conditions?

Yes, we think that an adequate number of volunteers was used in this study. Although not stated explicitly in the proposal, we used a nomogram [Carter, R.C. et al., *Human Factors*, 1981, **23**, 587-591] to estimate the required sample size for our psychological measures. For our dependent measures, values from the nomogram suggested 14-18 subjects would be required for most measures to produce statistically significant differences. We specified 25 volunteers anticipating that we might not get complete data for all volunteers on a given measure. The fact that no volunteers terminated during any of the conditions tested helped increase the power of our measures.

We recognize that "the anticipated effects" which were the basis for the nomogram's minimum number of volunteers are, at best, an approximation. Since we planned to use female volunteers that were relatively lean ($\leq$27% body fat), we expected their thermoregulatory response to be somewhat like that for male volunteers. However, we did not see the decreases in core temperature in our female volunteers that we expected. This may be due to a gender difference in cooling caused by differences in surface area, mass, and limb geometry [Seo, C.

UMI Dissertation Information Service, 1996]. Also, we used data from a Navy study employing a slightly colder temperature with a shorter exposure [Shurtleff D. et al., *Pharmacol Biochem Behav*, 1994, **47**(4), 935-941].

2. This project deviated from its Statement of Work (SOW) when it exposed volunteers to a cold air rather than a cold water stressor (without approval). Would cold water be more stressful? How might this change in procedure affect the outcome of this project?

When the proposal was written, the PI consulted with colleagues with physiological and thermoregulatory expertise and sought assistance with specifying temperature conditions, duration of exposure, and selection of volunteers. After the proposal was funded, the collaborators for this project reviewed the proposal very closely and decided the cold air paradigm offered many advantages over water. These included: It is easier to test and monitor several volunteers simultaneously, cold water might cool volunteers too rapidly so there would be insufficient time for psychological testing, cold water immersion requires more elaborate procedures to ensure the safety of volunteers, and water immersion would require modifications to the laptops to protect them from damage by water. Although we were strong on justification and rationale for using a cold air stressor, we were short on approval in making this change to the SOW.

Given equivalent temperatures, water would be far more stressful than air because of its greater thermal transfer properties. Since one wants sufficient exposure for people to be adequately stressed and enough time to assess these effects subsequently, it was easier to choose accepted temperatures with a cold air stressor so that volunteers would likely endure the full 90 min exposure. A more relevant question might be, "If our exposures to cold air were more severe would these more stressful conditions have affected our results positively?" As mentioned in the report, we can not answer this question from these or other data. However, the study by the Navy with males showed that tyrosine was efficacious with an air stressor at 4°C [Shurtleff D. et al., *Pharmacol Biochem Behav*, 1994, **47**(4), 935-941].

Literature, appearing after our study, confirmed that when men and women are tested together in cold air conditions, men lose more heat and experience greater decreases in body temperature than women [Seo, C. UMI Dissertation Information Service, 1996]. It is possible we may have overestimated the stressfulness of our cold air environment on female subjects because of this gender difference in thermoregulation and the scarcity of data from studies where female and male volunteers are studied together with the same conditions.

3. The Navy did a study with lightly-clothed males at 4°C and demonstrated a significant treatment effect for tyrosine on a memory task. In lieu of this finding in the Navy study, why did we use air conditions at 7°C?

We wanted to test with multiple dependent measures so the session duration had to be sufficient to allow time for the effects of stress on the volunteer and then time for testing. So, the durations of the exposures were different in the two studies. Our exposure at 7°C was for 90 min; the Navy's study was at 4°C for 60 min. We also note that the difference in thermal gradients, and thus environmental stress, between a 7°C and 4°C air environment are practically negligible and physiologically unimportant. Thus, it appears that other variables, besides the temperatures of the cold air, are responsible for the outcomes in the Navy's and our studies.

4. One finding from this study is that supplemental tyrosine and cold exposure resulted in a smaller, statistically significant, decrease in norepinephrine (NE) than did the combination of cold exposure and placebo. Is there additional information which will reconcile this unexpected finding?

The finding was contrary to what we would have predicted. Although this was a statistically significant difference ($p \leq 0.01$), Fig. 10 shows the average values with standard errors of the means and suggests that the difference in NE concentrations for these two conditions was small. It should also be remembered that these measurements are peripheral NE values.

ADDENDUM: COMMENTS TO ISSUES OF THE REVIEWER AND COR

To rule out possible biases in using the mean as the measure of central tendency and comparing conditions with differing Ns, we noted that the medians gave values similar to the means. When we only compared volunteers (N=11) with complete data for NE, mean and medians values were similar to the original values in Fig. 10. We were surprised by the finding originally but these statistical manipulations suggest it is robust.

We noted originally in the Results section (p. 17) that drawing blood samples from indwelling catheters in women participants after 90 min of cold exposure proved difficult and that extra variability in our measures may have resulted from the occasional, increased stress caused by the difficulty in collecting samples from some of our volunteers. We do not know if this contributed to this finding, but we can not rule it out.