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THESIS

SUPPLEMENTAL TYROSINE AND VIGILANCE PERFORMANCE IN A NORMOXIC HYPOBARIC ENVIRONMENT

Submitted by

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Department of Physiology

In partial fulfillment of the requirements

for the Degree of Master of Science

Colorado State University

Fort Collins, Colorado

Spring 2001

Supplemental Tyrosine and Vigilance Performance in a Normoxic Hypobaric Environment

Ryan W. Maresh, Captain, USAF, 2001
Department of Physiology, Colorado State University
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Exposure to stress results in a decrease in mental performance resulting from a depletion of catecholamines. This decrease can result in an increase in error rates, decreased decision making ability, mental confusion, and an overall decrease in cognitive performance. Previous studies have shown that supplemental tyrosine prevents the normal decrease due to stress. Tyrosine works by increasing the catecholamine precursor.

Nine subjects were exposed to a 3-hour protocol for 4 different exposures. During each exposure, the following stressors were used: 1) hypobaric environment (for two of the four exposures); 2) immobilization; 3) prolonged inactivity; 4) behavioral and psychophysiological testing; 5) cool temperatures and low humidity; and 6) 71 dB(A) chamber environment. Subjects completed computerized vigilance and cognitive tests and a mood questionnaire, each separated by a period of inactivity. Prior to exposure, subjects ingested either 100 mg/kg body weight of a placebo or tyrosine.

Supplemental tyrosine improved response time in all of the tests where it was recorded, with all except one being significant. Motor function and overall mood also significantly improved with the use of tyrosine. As expected, no measurement showed a significant improvement in performance with supplemental oxygen alone. The use of supplemental tyrosine did not significantly improve cognitive performance. Results of the contrast sensitivity tasks were not as conclusive and no improvement was found in visual temporal acuity. The results of the present study confirm those of previous studies and support the view that supplemental tyrosine can improve vigilance performance under normoxic hypobaric conditions.

COLORADO STATE UNIVERSITY

March 29, 2001

WE HEREBY RECOMMEND THAT THE THESIS PREPARED UNDER OUR SUPERVISION BY RYAN W. MARESH ENTITLED SUPPLEMENTAL TYROSINE AND VIGILANCE PERFORMANCE IN A NORMOXIC HYPOBARIC ENVIRONMENT BE ACCEPTED AS FULFILLING IN PART REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE.

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ABSTRACT OF THESIS

SUPPLEMENTAL TYROSINE AND VIGILANCE PERFORMANCE IN A NORMOXIC HYPOBARIC ENVIRONMENT

Exposure to environmental or physiological stress results in a decrease in mental and physical performance. The less control the individual has over these stresses, the greater the decrement. The decrease in performance, particularly mental performance, results from a depletion of catecholamines within the brain. This decrease, especially in norepinephrine, leads to alterations in normal brain function and an increase in error rates, decreased decision making ability, mental confusion, and an overall decrease in cognitive performance. All of these are compounded the longer the individual is unable to remove the stress. Previous studies involving rats and humans have shown that supplemental tyrosine (levels above normal dietary intake) prevent the normal decrease in performance in stressed individuals. Tyrosine, the initial precursor in catecholamine synthesis, works by increasing the availability of the precursor and thereby slowing the rate of norepinephrine depletion.

Nine healthy subjects from the Colorado State University community were exposed to a 3-hour protocol that was repeated for 4 different environmental conditions. During each exposure profile, the following stressors were placed on the subjects: 1)

hypobaric environment of 21,000 feet (only for two of the four exposure profiles); 2) immobilization in the form of a confined space (4' x 3'), the restriction of movement for a period of three hours, and the use of a breathing hood; 3) boredom associated with periods of prolonged inactivity; 4) behavioral and psychophysiological testing; 5) cool temperatures and low humidity; and 6) a sustained 71 dB(A) noisy chamber environment. During the course of each exposure, subjects performed several computerized vigilance and cognitive tests and completed a mood questionnaire. Each test was separated by a period of inactivity. Prior to each exposure, subjects were given either 100 mg/kg body weight of a placebo or tyrosine in capsule form. The following comparison groups were analyzed to determine if there was improvement in performance with the use of supplemental oxygen or supplemental tyrosine: 1) Ground-Placebo vs. Ground-Tyrosine; 2) Altitude-Placebo vs. Altitude-Tyrosine; 3) Ground-Placebo vs. Altitude-Placebo; and 4) Ground-Tyrosine vs. Altitude-Tyrosine.

The use of supplemental tyrosine resulted in an improvement in response time in all of the tests where it was recorded (Psychomotor Vigilance Task, Code Substitution Task, and Pattern Matching Task), with all except one test (Scanning Visual Vigilance Task) being significant. Motor function was also significantly improved with the use of tyrosine as measured by the Tapping Speed Task. As was expected, no measurement showed a significant improvement in performance with supplemental oxygen alone. The use of supplemental tyrosine did not significantly improve cognitive performance when measuring the percent correct on the Code Substitution Task and Pattern Matching Task. This may have been due to the insensitivity of the tests for the test conditions. Overall mood, as determined by the Profile of Mood States questionnaire, also improved with the

use of tyrosine. The results of the contrast sensitivity tasks (Functional Acuity Contrast Test and the Scanning Visual Vigilance Task) were not as conclusive as the other measures and no statements can be made as to the benefits of tyrosine on improving visual contrast sensitivity. No improvement was found on the Simultaneity Task, which measured visual temporal acuity. The results of the present study confirm those of previous studies and support the view that supplemental tyrosine can improve vigilance performance under normoxic hypobaric conditions.

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Solum volamus

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CHAPTER 1

INTRODUCTION

The United States Air Force's high altitude reconnaissance program and the current aircraft, the Lockheed U-2, impose many stressors on pilots in the program. Many other types of aircraft and crews, both military and civilian, are also exposed to increasing levels of stress, primarily due to fatigue associated with long-duration missions that often cross multiple time zones. While the U-2 community shares those same stressors, it is also exposed to many that are unique to the aircraft and mission. Unlike most other airframes, the U-2 operates alone (one aircraft) in the upper reaches of the atmosphere, routinely above 70,000 feet, with only a single pilot. Operational stressors for the U-2 community include long duration missions (regularly 9+ hours in duration) and extended and unusual duty hours. Environmental stressors include pressure extremes from physiological exposure to a cabin altitude of 29,500 feet, mandatory flight equipment (full pressure suit), low humidity environment inside the pressure suit and respiratory system due to breathing dry, 100% oxygen from one (1) hour preflight to exiting the cockpit (which leads to dehydration), temperature extremes due to both the full pressure suit and cabin environmental control systems, and a tight, confined cockpit that severely restricts vision and movement. Other self-imposed stressors can include the

conscious decision to dehydrate to minimize the need to urinate, poor sleep habits, and variable nutrition habits that also contribute to a potential decrease in pilot performance.

Of particular concern is the risk posed by fatigue and the resulting breakdown in mental alertness and performance. Between January 1992 and September 1998, there were eight Class A (loss of life or aircraft, or damage to equipment and property of over $1 million) U-2 accidents. Five of the eight mishaps (62.5%) occurred at night or early morning. Two more accidents (not Class A) also occurred during this timeframe and both happened at night (21). While there were multiple causes for these accidents, decreased performance due to fatigue, long missions, and the environment all contributed. In a survey conducted by a U-2 pilot as part of a graduate research project, 63% of those responding said that they had inadvertently fallen asleep for 1-5 minutes while flying the U-2 and one respondent reported that he had fallen asleep for 6-20 minutes (21). When you consider the concept of microsleeps (brief periods of sleep lasting a few seconds to a couple of minutes), where the majority of people are not even aware of how long they are asleep or that they have even fallen asleep, this percentage could be much higher.

Unfortunately many of the stressors, such as the cabin altitude, full pressure suit, and 100% oxygen, represent a compromise between aircraft and mission requirements and life support requirements. As such, these stressors are unavoidable and unchangeable. The flying community can control the self-imposed stressors and has attempted to minimize them through individual responsibility and education, as well as through implementation of policies such as crew duty-day limitations and crew rest requirements.

The last stressor mentioned earlier, nutrition, has recently received quite a bit of attention within the U-2 community.

In the spring and summer of 1999, a renewed interest in the importance of nutrition resulted in an Air Force nutritional assessment of the U-2 community. One result was the creation of a U-2 pilot nutrition pamphlet designed to educate the pilots of their nutritional requirements and special considerations (67). A high-protein, low-residue diet has always been taught and stressed to minimize, and hopefully prevent, bowel irritability, gas expansion, and fecal material due to the inability to deal with defecation while wearing the full pressure suit. Past aerospace physiologists associated with the U-2 program have discussed the potential use of tyrosine in the existing "tube food", the in-flight meal that is a unique, high altitude ration item, to help improve mental and physical performance. The nutritional assessment indicated that tyrosine supplementation should be an area for further investigation. Currently, many pilots consume tube foods that are high in sugar 30 to 45 minutes prior to landing to provide a "sugar high" in an attempt to increase awareness and performance. Supplemental tyrosine has been shown to prevent performance decrements in individuals exposed to various types of stress, including cold and hypoxia. Although pilots in the U-2 are protected from hypoxia by breathing 100% oxygen, they are still exposed to multiple forms of psychological and environmental stressors. It is believed that tyrosine could be a more beneficial way of enhancing performance in the U-2 community by complementing proper nutrition and sleep habits, stress management techniques, and the current physiological training requirements.

It is thought that the use of supplemental tyrosine placed in the existing tube food will help to reduce the challenges pilots face and provide a more effective way of dealing with performance decrements than loading up on sugar to get a "sugar high." While the benefits of tyrosine have been well documented, it is not known what effect supplemental oxygen will have when used in conjunction with 100 mg/kg supplemental tyrosine. To my knowledge, no studies have been conducted to look at the combination of tyrosine and supplemental oxygen. Oxygen may enhance the benefits previously demonstrated by the use of supplemental tyrosine or it may have no effect at all. If it is shown that tyrosine is still effective when used with 100% oxygen, it may be beneficial to the U-2 program as a supplement.

Hypothesis and Sub-Hypotheses. The purpose of this study was to investigate the effects of tyrosine supplementation on individuals breathing supplemental oxygen at simulated high altitude. This study tested the hypothesis that breathing supplemental oxygen while exposed to a hypobaric environment enhances the beneficial effects of supplemental tyrosine on vigilance and cognitive performance. The following sub-hypothesis were also evaluated:

1. Supplemental tyrosine will improve vigilance and cognitive performance at ground level (Ground-Placebo vs. Ground-Tyrosine).
2. Supplemental tyrosine will improve vigilance and cognitive performance while breathing supplemental oxygen at altitude (Altitude-Placebo vs. Altitude-Tyrosine).

3. Supplemental tyrosine, in combination with supplemental oxygen, will improve vigilance and cognitive performance more than the use of tyrosine alone (Ground-Tyrosine vs. Altitude-Tyrosine).
4. Supplemental oxygen alone at altitude will improve vigilance and cognitive performance (Ground-Placebo vs. Altitude-Placebo).

CHAPTER II

REVIEW OF LITERATURE

Stress and Catecholamine Synthesis

Stress. It is a part of everyday life that we all deal with differently. While some stress actually helps to improve our performance, stress that is too intense, or that we are exposed to for extended periods of time, causes our performance to suffer. Stress can be both mental and physical, with mental stress occurring when we are overburdened, task saturated, performing tasks that require extreme concentration, or there is a threat (either real or perceived) of being harmed. It can diminish our ability to make accurate, timely decisions, leads to confusion, and in the flying environment it can lead to loss of life or aircraft. Physical, sometimes referred to as environmental, stress results from exposure to extreme temperature, prolonged loud noise, immobilization, fatigue, exercise, and physical damage to our bodies and can have the same effects as mental stress on our ability to make decisions. Regardless of how successfully we outwardly deal with it, stress stimulates a physiological response from our bodies that can have adverse effects if not dealt with properly.

Stress can be divided into either short-term stress or long-term stress. Long-term stress causes more long-term changes, while short-term stress affects the minute-to-minute changes in metabolism (64). The autonomic nervous system responds to short-term stress through the release of the catecholamines epinephrine and norepinephrine.

Epinephrine plays an important role in mobilizing specific pathways to increase the conversion of stored energy sources into glucose (64). This is important to ensure that the brain has an adequate supply of fuel to properly function. As will be discussed, when catecholamine levels are depleted, normal brain function is altered and can result in an increased error rate, decreased decision making ability, mental confusion, and an overall decrease in cognitive performance.

One method of attempting to minimize the effects of stress without the use of pharmacological agents has been to enhance nutrition. While it has long been known that an adequate amount of calories, carbohydrates, proteins, fats, vitamins, and minerals is essential in maintaining health, our immune system, and performance, there is a growing interest in nutritional supplements. From a military application standpoint, nutritional supplements, if proven to be beneficial, reliable, and safe, provide a better alternative to pharmacological agents. The use of drugs to enhance performance and reduce fatigue presents the possible risk of side effects and potential addiction. Nutritional supplements are promising because of the relative lack of such risks. One possible supplement is the catecholamine precursor tyrosine.

The catecholamines epinephrine, norepinephrine, and dopamine are synthesized in the adrenergic neurons of the central nervous system, in the cells of sympathetic ganglia, and in the adrenal chromaffin cells in response to stress (19, 55). Additionally, when not stressed, their release occurs in a diurnal manner. Epinephrine tends to have a definite pattern of release that peaks in the early afternoon while norepinephrine is less stable with a less pronounced circadian rhythm (2). Interindividual differences also occur between morning-type and evening-type personalities. Catecholamine levels tend to be

lower in the morning and higher in the evening in evening-types while morning-types are just the opposite (2). Performance and alertness generally correspond to the peak periods, regardless of whether the peak occurs due to a diurnal rhythm or is stress induced (2, 30, 66, 72, 73). De Moja *et. al.* have reported that when a general state of arousal exists (i.e., when catecholamine levels are elevated), tactile discrimination, visual perception on easy tasks, and learning and memorization performance is enhanced (18).

Mammalian brains synthesize the catecholamines from the amino acid precursor tyrosine, a large neutral amino acid found in dietary proteins (6, 32, 55, 82). Figure 1 shows the biosynthesis pathway.

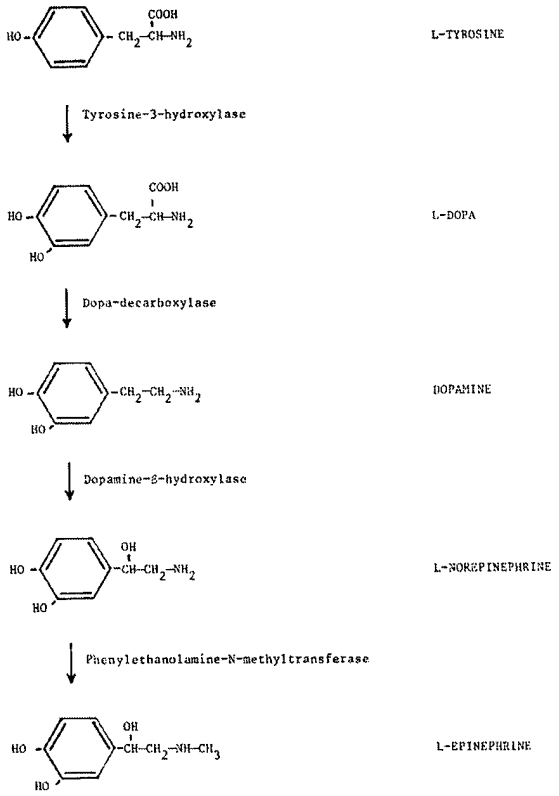

Figure 1: Catecholamine Biosynthesis Pathway (55)

The synthesis of catecholamines is regulated to meet the physiological demands of the individual. The enzyme tyrosine hydroxylase is the rate-limiting step in the biosynthesis of catecholamines and its activity appears to have a half-life of approximately three days (11, 32, 44, 57, 61, 64, 97). Alterations in the activity of adrenal tyrosine hydroxylase appear to vary depending on the type and duration of physiological stress (28). During normal conditions, tyrosine hydroxylase is only 50% - 75% saturated (31, 69). According to Mandel *et. al.*, there are at least two different mechanisms by which catecholamine synthesis is adjusted: feedback inhibition or induction of tyrosine hydroxylase. In feedback inhibition of tyrosine hydroxylase, the presence of catecholamines and DOPA inactivates the enzyme (55). Induction increases tyrosine hydroxylase activity by increasing enzyme protein synthesis through the up-regulation of the tyrosine hydroxylase gene in response to stress (55, 71, 81). Studies have indicated a 4.3-fold increase in the adrenomedullary and a somewhat smaller increase in the brainstem tyrosine hydroxylase mRNA following cold stress; however, there is not uniform stimulation of peripheral and central adrenergic systems (88). Cold stress also does not reduce the degradation rate of the enzyme (11). Tyrosine hydroxylase activity is increased in rats following repeated immobilization stress, most likely in response to the stress-induced depletion of the catecholamines (70). The regulation of tyrosine hydroxylase is due to the presence of a small, localized pool of catecholamines within the cytoplasm that act as an important regulatory factor in the biosynthetic process (55). Lehnert *et. al.* have also reported that a catecholaminergic neuron's firing frequency can be coupled to its precursor responsiveness through the phosphorylation of tyrosine hydroxylase (45). In this manner, the enzyme's affinity for

its cofactor is enhanced and becomes independent of the end-product inhibition and dependent on the availability of the precursor tyrosine.

There is a constant turnover of the catecholamines in the central and peripheral nervous systems, with an average half-life of 2 hours for dopamine, 2.5 hours for epinephrine, and 4 hours for norepinephrine (7, 55). The turnover rates vary from region to region, and when individuals are placed in a highly stressful situation or environment, catecholamine turnover increases and often synthesis does not keep pace with neurotransmitter release (6, 70, 83). One reason for this is because of the stress-induced elevation of glucocorticoids, which act to regulate "the synthesis and degradation of enzymes involved in the formation of catecholamines" (92). Frequent and rapid firing of catecholamine releasing neurons may enhance the activity of tyrosine hydroxylase and cause the enzyme to be more susceptible to control by the amino acid or may simply deplete the tyrosine pool within the nerve terminal (51).

In addition to the rate-limiting enzyme tyrosine hydroxylase, the rate of catecholamine synthesis is also directly related to the amount of the precursor tyrosine available (32, 60, 101, 102). By increasing the precursor supply, past studies on both rats and humans have found increased brain tyrosine and catecholamine levels.

It also appears that tyrosine affects not only catecholamine synthesis and turnover rate, but also the release of brain norepinephrine (33). Tyrosine can amplify neurotransmission selectively by increasing it at some synapses while having no effect at others (102). However, catecholamine-releasing neurons are responsive to tyrosine only when they have been, or are, very active (48, 60). The most successful method of increasing the brain tyrosine levels is by using pure tyrosine supplementation rather than

whole protein (32, 56). The most likely reason for this is that when whole protein is consumed, tyrosine must compete with the other amino acids for transport across the blood-brain barrier. The presence of these other neutral amino acids, and the resulting inhibition of tyrosine transport, interfere with catecholamine synthesis (32, 59, 102).

As presented above, tyrosine is essential for the synthesis of the catecholamines. The synthesis of catecholamines is part of the metabolic transformation that is the conversion of tyrosine to low-molecular weight compounds such as thyroxin and melanin (82, 98). Plasma tyrosine can also undergo at least two other kinds of metabolic transformation: 1) it can be taken up in the tissues and incorporated into peptides and proteins and 2) it can be deaminated to form ρ-hydroxyphenylpyruvic acid, which is a substrate for gluconeogenesis (98). Tyrosine serves as an energy source when catabolized through ρ-hydroxyphenylpyruvate to fumarate and acetoacetate (82). While not intending to minimize the importance of tyrosine in the synthesis of the thyroid hormones and melanin, its role is only mentioned to demonstrate tyrosine's importance in multiple pathways. The focus of this paper is on its importance in catecholamine synthesis and release in response to stress.

As has been shown, the synthesis of the catecholamines is dependent on the amount and availability of tyrosine and the activity of tyrosine hydroxylase. With the onset of stress, the catecholamines are depleted and the result can be a decrement in mental performance and a decreased resistance to numerous types of stress. Tyrosine supplementation has been studied by several military and other agencies as a potential nutritional supplement to help minimize these decrements and to possibly enhance performance and resistance to stress.

While tyrosine has shown promise in a number of studies, one key factor is required: tyrosine supplementation only works when there is a localized deficiency due to the individual being stressed (51, 60). When stressed, the increased neuronal activity causes the conversion of tyrosine to catecholamines at a faster rate to replenish the catecholamines that are released (45, 83). In normal, unstressed individuals, the addition of tyrosine has not been shown to improve or enhance performance and excess tyrosine is not converted into a catecholamine reservoir, but rather is excreted (83). It does not exhibit a stimulant effect, such as caffeine, when taken and therefore has not proven to be very effective in situations that cause a decrease in performance, such as boredom or sleepiness (51). In studies looking at sleep loss, tyrosine supplementation showed only non-significant improvements in counteracting performance decrements during situations that involved sustained, stressful work coupled with sleep loss (62). It is not known how effective tyrosine might be in extremely sleep-deprived and fatigued individuals. One promising aspect of the use of tyrosine to combat stress is that since it is readily found in common foods, there are no side effects to its use, and it is unlikely to have any long-term toxicity (6, 51, 62, 83). The primary reason for this is because, as a food constituent, tyrosine does not "interact directly with the synaptic macromolecules or accumulate *in vivo*" (47). Since the effects appear to be system specific and present only when a catecholamine deficit exists, the actions of supplemental tyrosine may be more specific than most drugs, but also less potent (47, 51).

Any kind of stressful environment can deplete catecholamine levels. In animal studies, rats have been exposed to cold, tail-shock, immobilization, water, and altitude. Rats that have been acutely stressed generally appear to be unable to function and both

complex and survival behaviors are depressed (51). These studies and the effects of tyrosine will be discussed later in the paper.

Dietary Requirements

Few food constituents have been evaluated for possible effects in modifying or enhancing performance and behavior, primarily because of the effectiveness of the blood-brain barrier at preventing all but a relatively small number from crossing and affecting the brain (48). Those constituents most studied include carbohydrates, proteins, caffeine, and a few amino acids. Caffeine has been extensively studied for decades and has been shown to have the properties of a stimulant and can enhance performance when stressed; however, there can be side effects when taken in large doses (48). One of the amino acids that has been studied as a possible aid in combating and minimizing the negative effects of stress (environmental, physical, or psychological) is tyrosine. Tyrosine has been shown to be beneficial and lacks the potential side effects of caffeine.

Tyrosine is a large, neutral non-essential amino acid that is necessary for proper maintenance and function of numerous organ systems. It is a constituent of dietary protein and is found in various quantities. Dietary values vary depending on the types and quantity of food ingested. Nutritional requirements are approximately 1-2 g/day, with normal dietary values ranging from 1 mg per 100 grams of white grapefruit juice to 2320 mg per 100 grams of parmesan cheese (63). Increased dietary protein raises the tyrosine blood plasma level and subsequently increases the brain tyrosine levels as the result of facilitated diffusion (26, 32, 33, 60). This pathway is shared competitively with other large, neutral amino acids, and the amount of tyrosine that actually enters the brain varies with the plasma tyrosine ratio (the ratio of the plasma tyrosine concentration to the

summed concentrations of other large neutral amino acids which compete for transport across the blood-brain barrier) (47, 103). The composition of a meal can affect the fluctuation of plasma tyrosine levels by at least two mechanisms: 1) by directly contributing a portion of the amino acids present in the dietary protein, and 2) by stimulating the secretion of insulin, which facilitates the passage of amino acids from the blood into tissues (26). Simply increasing the protein content of a meal will continue to increase the plasma tyrosine levels, but actually decreases the movement of tyrosine into the brain due to this competition (32). Supplemental tyrosine is more effective in increasing brain tyrosine levels because of the decreased competition.

Just as normal dietary values depend on the types and amount of food eaten, normal tyrosine levels in the human body vary between tissues and fluids. In addition, concentration levels vary between the sexes, being slightly higher in males. Numerous studies have been conducted to examine the amino acid concentrations in human blood plasma, cerebral spinal fluid (CSF), urine, and the rat brain. Most studies have quantified numerous amino acids in fasting adults and therefore minimized the variation in concentrations due to dietary intake.

Tyrosine CSF concentrations in the healthy, fasting adult range between 7.9 and 10 μM (27, 37, 59, 60, 75). As with brain levels, CSF levels change in response to plasma levels, which are normally between 50 and 70 μM (27, 37, 38, 54, 60, 75, 89, 94). Daily levels of tyrosine excreted in the urine range from 49 to 81 mg/day. While whole-brain tyrosine levels in humans are difficult to measure, whole-brain tyrosine levels in the rat have been reported to be between 47 and 65 nmoles/g in fasted rats, and between 47 and 173 nmoles/g in non-fasted rats (60). Rat brain tyrosine levels peak one hour after

oral ingestion and return to baseline levels after four hours (16). As little as 50 mg/kg of supplemental tyrosine have caused an 81% increase in brain tyrosine in rats 45 minutes after administration (100). Plasma levels are cited in the discussion on diurnal variation and tend to vary based on diet and time of day. Plasma levels have been shown to remain elevated above baseline values for over 4.5 hours. Banderet and Lieberman reported human plasma tyrosine baseline values of 42 ± 3.3 nmoles/ml and following administration of 100 mg/kg of supplemental tyrosine, plasma levels were 108 ± 5.1 nmoles/ml after 150 minutes and still elevated at 98.6 ± 6.3 nmoles/ml after 265 minutes (6). While plasma levels increase with increasing amounts of dietary protein, CSF levels will rise slightly until increased competition from other large neutral amino acids inhibits tyrosine transport across the blood-brain barrier. Likewise, urine levels increase if the excess tyrosine is not required.

Diurnal Variations

In addition to variation caused by the type and amount of food consumed, tyrosine levels display characteristic diurnal fluctuations. Numerous studies have been completed looking at the diurnal variation in plasma tyrosine levels and the effects of diet. Wurtman *et. al.* found that plasma tyrosine concentrations vary markedly each day in normal humans, independent of cyclic ingestion of protein or from exercise. The researchers fed volunteers a normal diet containing 0.71 g protein/kg and approximately 30 calories/kg, four times a day, and blood samples were collected every 3-5 hours over a 24-hour period. A temporal variation in tyrosine levels occurred in all individuals, with a peak (16.2 ± 0.82 μg/ml) at 1030 hours. Tyrosine levels then gradually declined to

intermediate levels over the next several hours until around 2100 hours when they fell sharply to a low (9.5 ± 0.35 μg/ml) at 0130 hours. The peak and low values for the volunteers on a low protein diet (2.7 g/day for 14 days) were slightly lower (12.7 ± 0.44 vs. 7.8 ± 0.31 μg/ml) and they occurred approximately 2-2.5 hours earlier, but the ratio of peak concentration:low concentration remained nearly 2:1 regardless of the amount of protein consumed (98). The daily low is most likely due to the secretion of insulin that facilitates the uptake of tyrosine by the tissues and thereby lowering the blood plasma levels (26).

Other studies have confirmed the daily fluctuations, but with slight differences in the concentrations and time of peak/low onset (12, 24, 25, 26, 56, 99). The most dramatic shift occurred when volunteers were fed a high-protein diet (150 g/day). The result of this high-protein diet was a substantial shift in the rhythm, with the peak occurring at 2300 hours and the low occurring at 0700 hours (26). The studies demonstrate that, while the amount of dietary protein does influence the tyrosine level, tyrosine's daily variation is determined more by an intrinsic mechanism. In addition, the diurnal tyrosine rhythm, alone, accounts for a quarter of the amplitude of total amino acid rhythm (98). The results of these studies are summarized in Table 1.

Table 1. Summary of Diurnal Variation Studies of Plasma Tyrosine Levels

Reference	Amount of Protein	Time of Peak	Peak Concentration	Time of Low	Low Concentration	High:Low
98	0.71 g/kg	1030	16.2±0.82 μg/ml	0130	9.5±0.35 μg/ml	1.7
	2.7 g/day	0830	12.7±0.44 μg/ml	2200	7.8±0.31 μg/ml	1.6
12						
Humans		1200		Midnight		
Rats						
(ad libitum)		0500		2300		
(0800-1200)		1100		0800 (declined from 0200)		
99	0.04 g/kg	0830	0.05 μmole/ml	2200	0.02 μmole/ml	2.13
	0.71 g/kg	1030	16.9 μg/ml	0200	9.9 μg/ml	1.7
	1.5 g/kg	1330-2130	0.09 μmole/ml	0200	0.05 μmole/ml	2.01
4	0 g	0700	56 nmole/ml	0300	30 nmole/ml	1.9
	75 g	1100	68 nmole/ml	0300, 1100	48 nmole/ml	1.4
	150 g	2300	95 nmole/ml	0700	56 nmole/ml	1.7

1 μg (or μmole) is equal to 1000 ng (or nmole)

Typical Dosages Used

In studies examining the use of tyrosine in combating stress, the majority have used pure tyrosine as a supplement at levels above that which individuals get in their normal diet. In human studies, the standard dose is 100-150 mg/kg administered in either a capsule form or in a drink. This dosage represents approximately 6-12 g of supplemental tyrosine, well above the nutritional requirements of 1-2 g/day. Generally, 100 mg/kg represents approximately 80% of an adult's daily dietary intake (6). One study conducted by Deijen *et. al.* used tyrosine in naturally occurring protein form because of dose and dosage form restrictions placed on them (16). In this study, researchers used orange juice with 70 grams of the diet powder PROTIFAR dissolved in it. This provided 2 g of tyrosine along with other amino acids for a total of 42 g of protein. Even with this restriction, they were still able to conclude that there was a benefit.

A typical dose of 100 mg/kg of supplemental tyrosine causes plasma tyrosine levels to peak approximately two hours after administration and to remain elevated for 4-8 hours (6, 15, 20, 36, 62, 90, 91, 101).

Hazards of Supplemental Tyrosine

As mentioned earlier, there are no known hazards associated with high levels of tyrosine in the diet, or with tyrosine supplementation, for normal healthy individuals (6, 50, 61, 82).

Benefits of Tyrosine Supplementation

Tyrosine has repeatedly been shown to be beneficial in a variety of stressful environments in both animal and human studies. It has been well known and well documented that exposure to altitude and the decreased partial pressure of oxygen available leads to performance decrements, both physiologically and psychologically (5, 86). Physiologic effects range from shortness of breath and fatigue to acute mountain sickness. Psychological effects range from altered mood states (ranging from depression to euphoria) to decrements in learning and memory. The number, type and severity, rate of onset, and duration of effect vary among individuals, as well as among exposures. All are related to the exposure altitude, how fast an individual reaches that altitude, and duration of altitude exposure.

Altitude-induced hypoxia and cold have proven to be effective environmental stressors and have been used repeatedly to study memory and learning. Banderet and Lieberman exposed volunteers to two levels of environmental stress: 1) 15°C and 4200 m

(450 torr) simulated altitude, and 2) 15°C and 4700 m (421 torr) simulated altitude. Results were compared to a control environment of normal temperature and pressure [22°C and 550 m (710 torr) altitude]. All exposures lasted 4.5 hours and all subjects were tested with placebo and 100 mg/kg supplemental tyrosine. They demonstrated that tyrosine decreased the adverse behavioral effects produced by exposure to cold and hypoxia, decreased the symptoms of headache, "coldness", distress, fatigue, muscular discomfort, and sleepiness. Subjects also displayed fewer adverse emotions, such as confusion, unhappiness, hostility, and tension, normally associated with exposure to environmental stressors (6). Additional studies have confirmed and extended these findings (49, 87). Rat studies have confirmed these results and also shown that tyrosine treatment reverses working memory escape latency decrements and alleviates hypoxia-induced retardation of learning (50, 85).

Tyrosine has also been shown to restore behavior and performance in rats with cold-induced stress decrements. Exposure to cold accelerates the firing of neurons that release norepinephrine. The increased firing rate eventually leads to the depletion of norepinephrine while also activating tyrosine hydroxylase, thereby making it more sensitive to tyrosine. The most dramatic changes occur in tyrosine hydroxylase mRNA levels, with cold exposure causing a 3-5-fold increase in mRNA levels within 3-6 hours of exposure (41). This further supports the findings of Stachowiak *et. al.* presented above. Supplemental tyrosine, either given for several weeks as a dietary supplement or immediately prior to the cold stressor, has been shown to protect animals when stressed (78). When rats are exposed to a cold environment and their body core temperature drops, they display behavioral depression. When core temperature was lowered to

between 30°C and 37°C, rats placed in 17°C water would swim attempting to escape for approximately 60 seconds and then assumed an immobile posture. Rats that had been pretreated with 400 mg/kg of tyrosine 30 minutes before the hypothermia treatment showed significantly decreased immobility in the swim test compared to those rats that received a saline pretreatment (78). Brady *et. al.* also showed that tyrosine protects against the effects of hypothermia when rats are placed in 2°C to 6°C water without prior cooling of body core temperature. They found that tyrosine prevented the stress-induced decrease in aggressiveness in both young and aged mice (9). Luo *et. al.* further supports the benefits of tyrosine on cold exposure (53). These studies have shown that tyrosine reverses the behavioral depression associated with hypothermia and can be an effective countermeasure against environmental stress.

When rats are exposed to acute, inescapable stress, they display neurochemical and behavioral changes such as learned helplessness and neurotransmitter-related motor deficits (91, 96). When Lehnert *et. al.* subjected rats to immobilization and electric tail shock, locomotion, hole-poking, standing on the hind legs (rearing), and exploration were all reduced when the rats were placed into an open-field/hole-poke apparatus 15 minutes after being stressed (46). When the rats were fed a tyrosine-enriched diet, these forms of behavioral depression were not displayed and the rats lacked the stress-induced depletion of norepinephrine of the control group (46). Reinstein *et. al.* further support these results (79, 80). Lehnert *et. al.* found that, in rats fed a normal diet, stress reduced the plasma levels of the six neutral amino acids measured. Tyrosine supplementation significantly increased both the plasma tyrosine levels and the brain tyrosine levels. Their results further supported the finding that tyrosine supplementation has no effect on unstressed

rats, but blocks the depletion of catecholamines in the brain when stressed (46). Gibson *et. al.* showed similar results with mice using the inescapable stress of being placed in 23°C water (34). These results also support the conclusions made from the cold-induced studies presented above.

Another area where tyrosine has been shown to have beneficial effects is the cardiovascular system. The cardiovascular system responds to input from the nervous system through receptors for the catecholamines norepinephrine and epinephrine. Depending on where in the cardiovascular system stimulation occurs and the type of receptor activated, sympathetic tone is either increased or decreased. This results in either an increase or decrease in heart rate and/or blood pressure. Hypertension stimulates the norepinephrine-releasing neurons in the brainstem to increase their firing frequency and the release of norepinephrine acts in an inhibitory manner to suppress the activity of other neurons (102). This ultimately decreases the activity of the peripheral sympathetic neurons and the chromaffin cells of the adrenal medulla. With reduced catecholamine release at the periphery, vasoconstriction and cardiac output are reduced (102). Tyrosine appears to amplify this behavior within the brainstem since only those neurons are firing. Hypotension has the opposite effect, it raises blood pressure because the inhibitory neurons in the brainstem are suppressed and the sympathetic neurons and chromaffin cells are activated (102).

One study using dogs has even shown that tyrosine diminished the vulnerability of the heart to ventricular fibrillation, possibly reducing the risk of stress-induced cardiac arrest (84). In studies using normotensive and spontaneously hypertensive rats that were not stressed, tyrosine had a positive effect by lowering blood pressure. Rats were

injected with various doses of tyrosine and the effects on blood pressure were found to be dose dependent. The administration of 50 mg/kg tyrosine resulted in a slight reduction in blood pressure, but 100 mg/kg was the smallest dosage that consistently lowered blood pressure. The maximal effect was with a dose of 200 mg/kg, with increasing amounts of tyrosine having little affect on further lowering blood pressure. Regardless of the dose, the maximal effect occurred within two hours of administration and lasted for approximately 4-5 hours. The maximal dosage given, 400 mg/kg, lowered blood pressure in normotensive rats by 8 ± 1.5 mm Hg. In spontaneously hypertensive rats, the effect was much greater. The same dose of tyrosine (400 mg/kg) lowered blood pressure by 40 ± 6 mm Hg. This was only slightly greater than when using 200 mg/kg. The results of this study confirmed that tyrosine works to lower blood pressure by working within the central nervous system. This was shown by co-administering other large neutral amino acids that compete with tyrosine for passage across the blood-brain barrier. When tyrosine had to compete for uptake in the brain, it had little or no effect on reducing blood pressure (90). Other studies have shown that a diet containing 1% L-tyrosine can also lower blood pressure in spontaneously hypertensive rats (68).

Tyrosine has also been shown to raise blood pressure in hypotensive rats. Conlay *et. al.* examined the effects of tyrosine on hypotensive rats by bleeding two groups until they had lost 25% of their original blood volume (Group 1) or until their blood pressure dropped to half the original pressure (Group 2). Both groups received tyrosine dosages of 25, 50, or 100 mg/kg 45 minutes later. The response was dosage dependent, but in all cases the administration of tyrosine significantly raised systolic blood pressure within 5 minutes (13). Whereas Sved *et. al.* showed that tyrosine acts in the brain to lower blood

pressure, Conlay *et. al.* found that tyrosine acts on the adrenal medulla to raise blood pressure. They tested this by subjecting rats to both hemorrhage and bilateral adrenalectomy. In the adrenalectomized rats, tyrosine did not significantly raise blood pressure. However, in rats that were bled but not adrenalectomized, tyrosine caused a 38% increase in systolic blood pressure. The study also found that tyrosine's effect was diminished or blocked with the administration of other large neutral amino acids (13). In a similar study by the same researchers, they showed that tyrosine increases blood pressure by 60 ± 8% in rats made hypotensive by hemorrhage. They confirmed that the effects of tyrosine during hemorrhagic shock are due to tyrosine accelerating the synthesis of catecholamines in peripheral structures (14).

Human studies of the effects of tyrosine on blood pressure have showed many of the same results as those using rats. Most of the studies, however, have monitored blood pressure as a secondary parameter and not as the primary event. Deijen *et. al.* used military academy cadets to study the effectiveness of tyrosine on reducing "real-life" stress. They studied the psychological and physical stresses associated with participation in a two-week combat training course conducted as part of their regular training program. The subjects were given a drink containing two grams of tyrosine once a day for five days. The investigators observed that, in addition to improving memory and tracking skills, tyrosine reduced systolic and diastolic blood pressure by up to 14 mm Hg (16). A second study by Deijen and Orlebeke showed that diastolic blood pressure was significantly reduced 15 minutes after ingestion of 100 mg/kg of tyrosine (15).

A study by Dollins *et. al.* specifically examined the effects of tyrosine on healthy humans exposed to lower body negative pressure (LBNP) to determine what impact

tyrosine had on cardiovascular stress. In the study, adult males were exposed to a LBNP of –50 mm Hg, starting at –20 mm Hg and reduced by 10 mm Hg every three minutes until –50 mm Hg was reached. Subjects were then exposed to –50 mm Hg for 30 minutes or until presyncope symptoms were present. Results showed that 100 mg/kg of tyrosine reduced the physiological decrements normally associated with LBNP. Subjects displayed a higher pulse pressure and overall improved tolerance to LBNP (20). Tyrosine could be beneficial in aiding astronauts cope with the orthostatic intolerance they experience upon their return to Earth, but more research is needed in this area.

Tyrosine has also shown promise in protecting individuals from the long-term effects of stress. Ample evidence exists that stress influences the immune system and that uncontrollability of the stressor and the amount of effort involved in trying to cope with the stressor are two important factors that determine the level of physiological response to stress (76). One effect of stress is an increase in cortisol levels, which can have adverse effects on health. Levels can remain high, and can even be amplified, during post-stress periods. This can contribute to a suppressed immune system and the development of ulcers that are commonly reported in individuals who deal with chronic stress as part of their jobs or daily routines. Deinzer *et. al.* demonstrated that tyrosine is able to prevent some post-stress disturbances in rats. When rats were subjected to one hour of water restraint stress and then examined for gastric ulcers, those rats receiving tyrosine had fewer and smaller gastric lesions than the rats that received saline (17). The use of tyrosine, in addition to stress reduction and management, may prove to be effective in coping with the long-term effects of chronic stress; however, at this time more research is needed in this area.

Reinstein *et. al.* found that tyrosine tends to suppress the rise in plasma corticosterone in acutely stressed rats, while having no effect on corticosterone levels in unstressed rats. Their results suggest that supplemental tyrosine may be beneficial to individuals who are suffering depression due to high levels of corticosterone resulting from environmental stress (80). Lehnert *et. al.* further support the attenuating effect of tyrosine on stress-induced release of ACTH and corticosterone in animals (45). One possible reason is by increasing the synthesis of norepinephrine, which has been shown to decrease CRH and inhibit ACTH secretion (70).

Tyrosine shows potential in the treatment of depression and for some neurological diseases. Gelenberg *et. al.* have reported limited success in treating depression using 100 mg/kg of tyrosine in patients who had problems with their normal anti-depression medication (31). Tyrosine's potential benefits in reversing some of the initial neurological conditions associated with the early stages of Parkinson's disease is most likely due to increasing the synthesis and release of dopamine (57).

Another area that tyrosine has been shown to be beneficial, although somewhat limited, is in military operations where individuals are exposed to a high operational tempo. A high operational tempo is usually accompanied by individuals working long hours, irregular and rotating schedules, and being under difficult conditions such as deployment. Common problems associated with this condition are: physical and mental fatigue, effects of shift-work/jet lag, sleep deprivation, stress, anxiety, and performance and mood decrements. Numerous animal studies have shown that fatigue and sleep deprivation cause a depletion of brain norepinephrine and lead to mood and performance decrements (69). As shown in this paper, tyrosine can reverse these negative effects.

Despite the proven benefits of tyrosine, it has not been shown conclusively that it is an effective countermeasure against fatigue and sleep deprivation alone. To specifically study the effects of tyrosine on sustained operations, Neri *et al.* exposed volunteers to a moderate intensity (70 dB A), low-frequency (150 Hz cutoff) noise to simulate the muffled noise of an aircraft engine. The subjects performed a number of performance and mood tests during an extended nighttime period (starting at 1930 and ending at 0820) of 13 hours. All subjects remained awake during the day prior to testing so that they had a period of sustained wakefulness of at least 24 hours by the end of testing. Six hours into the experiment, half of the volunteers received 150 mg/kg of tyrosine and the other half received a placebo. The tyrosine group showed significant improvement in performance during the tracking task and fewer lapses on the running memory task. While individuals reported a reduction in sleepiness and fatigue, the findings were insignificant. The effects started approximately two hours after administration of tyrosine and lasted for roughly five hours. Their results suggest that tyrosine could be beneficial in combating high operational tempo and sustained operations (62). Similar conclusions are made by Salter (83).

One area where the use of supplemental tyrosine has not received any attention is during exposure to heat. Epstein *et. al.* reported that vigilance and complex cognitive tasks deteriorated when individuals were exposed to various heat loads ranging from 21°C to 35°C (22). They showed that the intensity of the task and heat load on deteriorating performance are synergistic and that even highly motivated subjects are affected by heat load.

The effects of heat load are of great concern when wearing the full pressure suit. The full pressure suit forms an airtight barrier around the pilot and with inadequate ventilation, core body temperature can rise very quickly. NASA has dealt with this problem through the use of liquid-filled cooling garments. However, the U-2 program stills relies on airflow to cool the pilot. On the ground, ventilation is provided by a hand-held unit that combines liquid oxygen, which is first converted to gaseous form, and ambient air in a 40:60 ratio. This cools the ambient air used to vent the suit by approximately 20°F. This is effective and acceptable until the ambient temperature gets too high and the unit is unable to adequately cool the air that is vented into the pressure suit. The aircraft ventilation system performs better at altitude but can lead to inadequate ventilation and cooling while on the ground and during transition work (low altitude flying such as approaches and pattern work). If taxi times are prolonged, heat build-up inside the suit can occur and pilot heat stress can become an issue. Globe temperatures in some operating locations can reach 135°F or more and temperatures inside aircraft cockpits have been recorded as high as 171°F (77). Supplemental tyrosine may help deal with the negative effects of heat on pilot performance and is an area for further research.

CHAPTER III

MATERIALS AND METHODS

Experimental Design

A two-fixed-factorial, fully nested design, with 2 x 2 levels (Altitude, Nutrition) and repeated measures across both factors was used. For the purposes of this study, the statistical analysis was simplified to make only four comparisons. The paired *t*-test, with appropriately adjusted p values, was used to make these comparisons. The Bonferoni adjustment was made to deal with the increased Type 1 error probability associated with multiple comparisons.

Exposure Profiles

To study the effects of tyrosine supplementation during hypobaric exposure and breathing supplemental oxygen, subjects were exposed to four (4) different environmental conditions according to exposure profiles listed below. Each subject completed all four (4) exposure profiles and all exposures were a maximum of three (3) hours unless stopped by the volunteer or researchers. Exposure Profile 1 provided a baseline to compare the effects on performance when supplemental oxygen was used alone and also the effects on performance when supplemental oxygen and supplemental tyrosine were used together. Subjects were briefed on the altitude of the exposure and the test conditions prior to each exposure. Each subject completed the two ground exposure

profiles first; however, not necessarily in order. The use of either tyrosine or placebo was randomly generated for each subject prior to the first exposure at ground level and the opposite treatment was given for the next exposure. The same procedure was used for the altitude profiles. The following four (4) exposure profiles were used:

1. Ground Level—Day
   Without Supplemental Oxygen
   Placebo
   Duration: 3 hours

2. Ground Level—Day
   Without Supplemental Oxygen
   Supplemental TYR
   Duration: 3 hours

3. FL210—Day
   Supplemental Oxygen
   Placebo
   Duration: 3 hours

4. FL210—Day
   Supplemental Oxygen
   Supplemental TYR
   Duration: 3 hours

The hypobaric exposures were to 21,000 feet to provide an adequate stressor to individuals who are already acclimated to approximately 4,800 feet, the elevation of Fort Collins, CO, where the testing was conducted. This altitude was selected to more closely reflect the cabin altitude of 29,500 feet for the U-2 during a high flight above 50,000 feet. A limit of 21,000 feet was set for safety concerns to minimize the possibility of decompression sickness. Research by Webb *et. al.* showed that the lowest altitude occurrence of decompression sickness was a 5% incidence at 21,200 feet for exposures with no pre-oxygenation (95). In addition, all altitude exposures were separated by a minimum of 48 hours. Following the guidelines in Air Force Instruction (AFI) 11-403, Aerospace Physiological Training Program, subjects were required to prebreathe 100% oxygen at ground level for 30 minutes to further minimize the risk of decompression sickness (1). Also following AFI 11-403, ascent and descent rates did exceed 5,000 feet

per minute (1). However, due to the capabilities of the chamber facility, rates never exceeded 2,000 feet per minute.

All procedures were approved by the Colorado State University Human Research Committee.

Medical Considerations

Prior to beginning research, an agreement was in place with the Colorado State University Hartshorn Health Service. Three physicians agreed to act as consultants for any medical questions that arose and to respond in the event of an emergency. All were briefed on the extent of the research and the protocol and toured the Hypo-Hyperbaric Chamber Facility prior to starting. One of the three physicians was notified prior to the start of each altitude exposure and again upon completion of the exposure.

Each subject completed an approved initial medical screening questionnaire, along with the consent form, before participating. A simple "How Are You" questionnaire was also completed prior to each exposure.

Chamber Environment

A hypo/hyperbaric chamber was used to simulate an altitude of 21,000 feet. The chamber was used at ambient temperature (66±2.5°F) and humidity (27±5.5%) and under normal chamber lighting for all exposure profiles. Subjects conducted the ground level testing inside the chamber to maintain the same environmental testing conditions as at altitude.

Subjects

A total of 9 subjects (4 males, 5 females; Age 23.7±3.8; 79.8±13.3 kg) volunteered for participation. All subjects were required to wear USAF flight gloves to simulate the decreased dexterity of wearing the full pressure suit gloves. No other clothing restrictions were placed on the subjects. Once inside the chamber, subjects were confined to a restricted amount of space (roughly 4' x 3') to simulate the confinement of the cockpit and were not allowed to stand or move around for the duration of the exposure.

Subjects were instructed to eat as they normally would prior to an exposure with no restrictions placed on the content of the meal. They were asked to abstain from consuming caffeine for 12 hours prior to each exposure.

Gas Supply Set-up

During each exposure, subjects received a continuous supply of either oxygen or breathing air delivered via a hood. The set-up for the hood is described below. Supplemental oxygen was used for exposures at 21,000 feet (provided an alveolar oxygen partial pressure of approximately 268 mmHg) while compressed breathing air was used for all ground level exposures. Two (2) 5-foot high pressure gas cylinders were used to ensure a continuous, uninterrupted supply of gas was maintained throughout each exposure. A 2-stage regulator was used on each cylinder to step down the cylinder pressure from 2200 psi to 60-90 psi. Each 2-stage regulator was connected to a single T-fitting using a shut-off valve. This allowed the researcher inside the chamber to isolate a single cylinder for use and to ensure easy and rapid switch over in the event one cylinder

ran low. A single high-pressure gas line was connected to a U. S. Air Force A-14 demand regulator, which was connected in series to a second A-14 regulator. The A-14 regulators allowed the researcher to select a range of supplemental pressures, if necessary, for the inside observer and the subject(s) independently of each other. This supplemental pressure provided a continuous flow of gas into the hood. When two (2) subjects were connected to the set-up, a separate, identical set-up was used for the researcher. Figure 2 represents the set-up that was used:

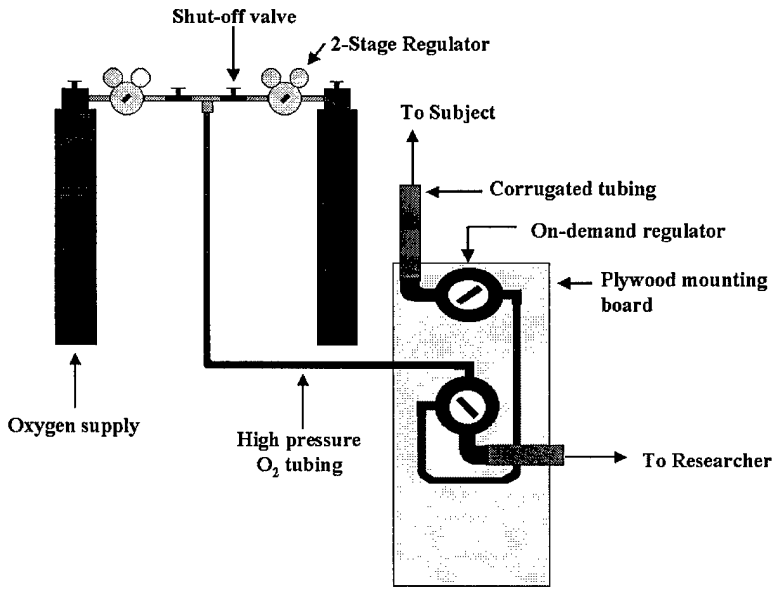

Figure 2: Gas Supply Set-up

Hood Set-up

The hood (Sea-Long Oxygen Head Tent, Sea-Long Medical Systems, Inc.) was a clear plastic bag that covers the head and has a latex neck seal that was trimmed to fit the volunteer's neck. It contained a visor in the front to improve vision while looking through the hood. The latex neck seal created an airtight seal and prevented ambient air from mixing with the supplemental oxygen while at altitude. The hood contains an inlet

port for the supply gas and an exhaust port to remove expired air, which was vented into the chamber. The hood was partially inflated by the supply gas to lift it off the volunteer's face. The hood was thoroughly cleaned between exposures using detergent and warm water and then wiped with 70% isopropol alcohol. In addition, each subject used the same hood and neckseal for all four (4) exposures.

The hood, supplied with compressed breathing air instead of supplemental oxygen, was used during exposures to ground level to maintain the conditions at 21,000 feet. A hood was used instead of a mask to simulate the full pressure suit helmet worn by pilots on high altitude U-2 missions. In addition, the hood helped to minimize any possible feelings of claustrophobia that a volunteer, who is not used to wearing life support equipment for extended periods of time, may experience from wearing a mask. Figure 3 represents the hood set-up that was used:

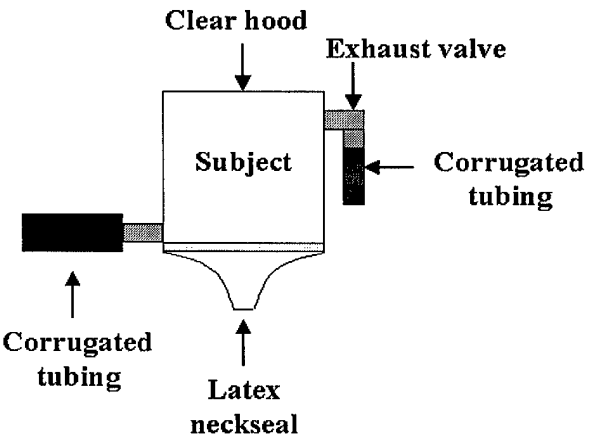

Figure 3: Hood Set-up

Inside Observer

Each exposure had one (1) researcher who acted as the inside observer (IO). The IO followed the same precautions (supplemental oxygen, preflight and post-exposure briefings, exposure restrictions, etc.) as the subjects and breathed oxygen supplied via a hood during the entire exposure to altitude. The IO was responsible for recognizing and handling any emergencies that occurred inside the chamber while at altitude. An "emergency procedures" checklist was available inside the chamber and the IO was in communication with the supervising individual outside the chamber who acted as the chamber operator and overall safety observer. An identical "emergency procedures" checklist was also available outside the chamber.

The IO was responsible for supervising the various tests that the subject(s) performed at altitude. They were trained to recognize the signs and symptoms of decompression sickness and hypoxia and monitored the subject(s) for any indication that a problem existed. If a problem occurred that could not be corrected at altitude, the IO terminated the exposure and initiated an immediate descent.

Tyrosine or Placebo Administration

Each subject was given 100 mg/kg of body weight of tyrosine (L-Tyrosine, Jo Mar Laboratories) or cellulose placebo (Clinical Nutrition Research Laboratory, Department of Food Science and Human Nutrition, CSU) taken orally in the form of a capsule upon their arrival at the chamber facility prior to the start of each exposure. The subjects were not informed as to whether they received tyrosine or placebo.

Cognitive/Vigilance Testing

In choosing tests to study the use of nutritional components such as tyrosine, the selection of the individual tests is extremely important. In addition, when doing environmental research, the tests chosen must be administered repeatedly in a baseline condition and in the new environment (10). Bittner *et. al.* state that "when following the time-course of performance in studies on vigilance, maturation, or environmental stress," it is useful to measure human performance capabilities repeatedly (8). These repeated measurements must be meaningful, and easily and clearly interpretable (8). A number of test batteries exist that have been successfully used by various researchers. For the proposed study, the selection of tests centered around the desire to study the effects of tyrosine on vigilance, mental performance, and mood in an individual exposed to a hypobaric environment while breathing supplemental oxygen. Since numerous studies have shown that tyrosine minimizes decrements in these functions in a hypoxic environment, the aim of this research was to determine if those same effects were present in a normoxic hypobaric environment. The one factor that was considered throughout the design and selection of tests was the requirement for the individual to be stressed or challenged. The current study was conducted using the following stressors: 1) hypobaric environment (exposure to 21,000 feet); 2) immobilization in the form of a confined space (4' x 3'), the restriction of movement for a period of three hours, and the use of the breathing hood; 3) boredom associated with periods of prolonged inactivity; 4) behavioral and psychophysiological testing; 5) cool temperatures and low humidity; and

6) a sustained 71 dB(A) noisy chamber environment due to the air conditioning unit inside the chamber.

It has been demonstrated that human performance oscillates on 1.5 hour cycles with performance tending to increase on one task with an accompanying decrease on another (43). Pepper *et. al.* also state that "environmental stressors which potentially affect performance are manifested as a function of duration of exposure" (43). Therefore, all tests (except the Scanning Visual Vigilance Test) were administered twice during each exposure and the order was varied to: 1) minimize any effects of this rhythm; 2) minimize the effects of expectation that may occur from providing the test in the same sequence each time; and 3) observe the effects over the duration of a three hour exposure. All computerized tests were run on a Gateway2000™ laptop computer.

Description of Individual Tests

Visual Contrast Sensitivity (Functional Acuity Contrast Test: FACT)

The physiological stimulus of light exposure can cause catecholaminergic neurons to become tyrosine-dependent (35). Gibson *et. al.* showed that exposure to environmental lighting of 350 lux (bright office lighting) selectively activates dopaminergic neurons and causes these neurons to become tyrosine-dependent. They state that "since treatments that activate tyrosine hydroxylase (TH) also cause neurons to synthesize more catecholamines after tyrosine administration, the most likely explanation for our findings is that, as a consequence of light exposure, retinal TH became activated and thus dependent on the availability of its amino acid substrate." They also demonstrated that the administration of 100 mg/kg of tyrosine increased retinal

dopamine's synthesis and release in the rat. The administration of tyrosine had no effect on rats that were maintained in the dark (35).

The FACT consists of a contrast chart with five (5) rows and nine (9) columns of diminishing visual contrast. The progression of the sine-wave grating size changes in steps equal to one octave between rows A, B, C, and D and a half octave between rows D and E. The corresponding spatial frequencies are 1.5, 3, 6, 12, and 18 cycles per degree. There is a 50% loss or 100% gain in contrast for any two contrast step increase or decrease respectively and the gratings are tapered into an average gray background to eliminate ghost images. The gratings are tilted +15°, 0°, and -15° to keep them within the orientation bandwidth of visual channels. Each grating patch is assigned a contrast value. The test was performed with the subjects holding the chart approximately 18" in front of their eyes.

The FACT was included to determine if tyrosine had any effect on improving visual contrast sensitivity. If so, this could be important in helping to enhance flight safety and improve the pilot's ability to discriminate between objects and information presented on maps under varying lighting conditions. Bittner *et. al.* also recommend this task for repeated-measures applications (8). This test was conducted once at the beginning and once at the end of each exposure.

Psychomotor Vigilance Task (PVT)

The catecholamines have been shown to affect the ability to perform mental work characterized by low energy expenditure and a sustained high level of attention (29, 65). O'Hanlon *et. al.* point out that elevations in the concentrations of the catecholamines

"may have beneficial effects on the capability for prolonged mental work since they appear to directly affect neural activity within arousal centers in the mesencephalic reticular formation" (65). The typical decline in vigilance in monotonous tasks is widely believed to occur because the areas of the brain that are responsible for maintaining vigilance gradually cease to respond as they become habituated to the repetitious occurrence of a given stimulus. O'Hanlon *et. al.* state that this loss may be prevented or delayed by an increase in the concentration of circulating epinephrine (65).

On a typical U-2 mission there are extended periods of time when the workload is low and sustained vigilance may be difficult, regardless of the pilot's motivation. Throughout the mission, the pilot is required to maintain a high level of attention to monitor aircraft and sensor systems. Providing more of the catecholamine precursor tyrosine may enhance the synthesis and release of catecholamines and help to keep vigilance elevated. The PVT provided a method of testing vigilance over the course of a three (3) hour chamber exposure during which time the volunteer had periods of time with relatively little to do.

The PVT requires sustained attention and discrete motor responses. It is sensitive to many minor cognitive stresses, including fatigue due to sleep loss, circadian variation, and shift work. Subjects were exposed to both cognitive and environmental stresses while inside the altitude chamber. The task was run for 10 minutes in the visual-only (0.5 inch LED) mode. The volunteer performed the PVT twice during each exposure, once at the beginning and once at the end.

The PVT is performed using the Vigilance Task Monitor, Model PVT-192. The monitor is a 8" x 4.5" x 2.4" portable, battery operated device that runs a simple reaction

time test for 10 minutes. The subject watched the digital counter display (0.5 inch LED) and turned off the counter as quickly as possible when it started. A relatively quick response is approximately 200 msec. The PVT-192 downloads its data in ASCII text format to a Pentium CPU based computer using dedicated interactive software. Data collected by the PVT includes the reaction time for each stimulus presented, each false alarm (any button press that occurs when the counter is not running), and each wrong response (pressing the wrong button).

Pattern Matching Task

Bittner *et. al.* state that pattern comparison "assesses an integrative spatial function neuropsychologically associated with the right hemisphere" (8). One of the most critical tasks of a pilot is to recognize if a problem exists and then to properly diagnosis what it is and how to properly deal with it. Often times the pilot is required to do so in a relatively short amount of time while under increasing levels of stress. Many times this is also accompanied by the requirement to deal with multiple tasks or procedures. The pressure of time and increasing workload can lead to attention management problems such as temporal distortion, channelized attention, inappropriate use of checklists, or simply misdiagnosing the problem. However, one method of successfully coping is to use a pattern-matching approach to deal with the problem instead of analytically attacking it. One test battery, the Performance Assessment Test (PATSYS) (Dr. Robert Kennedy, RSK Assessments, Inc.), is run on a lap-top computer and has been used in a wide range of studies that have dealt with the effects of vibration, simulator after-effects, flight tests, hypoxia, and drug effects and in environments such as

vibration platforms, ships, and hypobaric and hyperbaric chambers (39). The system has been used extensively with much success.

The successive pattern comparison task in the PATSYS tests visual pattern recognition and spatial memory by displaying a two random patterns of asterisks on the screen and the subject has to decide if the patterns are the same or different. Appendix A show a typical asterisk pattern displayed on the screen. The task ran for 3 minutes per trial and was conducted twice during each exposure. It is intended to test knowledge-based cognitive functions as opposed to rule-based or skill-based functions. Results from the Pattern Matching Task provided for comparisons between exposures with and without the use of supplemental tyrosine. It was hypothesized that performance would improve with the use of tyrosine by enhancing the synthesis and release of catecholamines in the brain.

Code Substitution Task

Bittner *et. al.* state that code substitution "is a mixed associative memory-perceptual speed task which provides for a traditional assessment" (8). Code substitution is a test of mental functioning and has been used repeatedly over the years as a method of testing intellectual speed and power (74). It is a mixed associative memory and perceptual speed test with visual search, encoding, decoding, and rote recall that requires solutions to problems posed in verbal-sequential order. It has been demonstrated to have adequate test-retest reliability and, based on laboratory and environmental results, the Code Substitution Task was recommended for environmental research test batteries (74).

A line of code is displayed across the top of the screen in two lines. The upper line shows nine letters from the alphabet, selected randomly. The second line shows the digits 1 through 9 in random order and associated with the letters in the first row. The screen also shows letters in a third row and marked blanks in a fourth row. The subject, working in the fourth row, fills in the number from the code that corresponds to the letter in the third row. The Code Substitution Task screen is shown in Appendix B. This task is a computerized test that runs as part of the PATSYS, runs for 3 minutes and was run twice during each exposure.

The data recorded by the task include the number of correct responses and the mean reaction time.

Simultaneity Task

The Visual Simultaneity Task (time domain) was included to balance the Visual Contrast Sensitivity Test (spatial domain). This visual function may be fundamental to the performance of jobs that require complex visual-motor activities (40). The Visual Simultaneity Task is a computerized test that runs as part of the PATSYS. It measures the briefest mean interval the volunteer can perceive between the appearances of two small square symbols on a computer screen. The locations of the squares are unchanging and are presented on either side of a central fixation point. The interval between the appearances is shortened until errors are made by the volunteer and then lengthened again. The cycle is repeated several times, with one trial lasting about one minute. Each volunteer completed the visual simultaneity task twice during each exposure, once early in the exposure and once again towards the end of the exposure. The Simultaneity Task screen is shown in Appendix C.

Tapping Speed Task

This task has been used historically to measure fundamental motor speed. It is a computerized test that is included in the PATSYS and runs for 20 seconds per session. The task is performed three times during each trial by alternately tapping two specified, unchanging keys on the computer keyboard as rapidly as possible using the non-dominant hand. There were two (2) sessions during each exposure.

Profile of Mood States

The Profile of Mood States (POMS) is a paper and pencil inventory of subjective mood states that consists of 65 mood-related adjectives on a five-point scale (4). Previous research has demonstrated that the adjectives factor into six mood sub-scales (Tension, Depression, Anger, Vigor, Fatigue, and Confusion) (4). The POMS was administered once at the end of each exposure with the subjects answering the question: "How have you been feeling during this exposure?" The POMS questionnaire is shown in Appendix D.

Scanning Visual Vigilance Test

The Scanning Visual Vigilance Test (SVT) is "a variable-length detection test designed to assess the ability of individuals to maintain visual alertness for sustained periods of time" and was "designed to be sensitive to changes in vigilance produced by subtle variations in performance, such as those produced by low doses of centrally acting food constituents, drugs, or environmental stress" (52). The SVT is a computerized test that randomly presents a simple, near-threshold stimulus at pseudo-random locations on a

computer screen and can run for up to two (2) hours (52). It minimizes the cognitive load and thereby emphasizes the vigilance aspects of the task.

The task was run using 150 stimuli appearing at random times spread out over 30 minutes. The data recorded from the task include the number of stimuli, the number of correct hits, the number of false positives, and the mean reaction time. It was run on a Gateway2000™ laptop computer for 30 minutes midway through each exposure.

Table 2 summarizes all of the tests and the types of measurement made during the present study.

Table 2. Summary of Tests and Test Measures.

Test	Type of Measurement
PVT	Vigilance Performance
SVT	Vigilance Performance
POMS	Mood
FACT	Spatial Contrast Sensitivity
Simultaneity Task	Vigilance Performance Temporal Contrast Sensitivity
Code Substitution Response Time Percent Correct	Cognitive Performance Speed Component Accuracy Component
Pattern Matching Response Time Percent Correct	Cognitive Performance Speed Component Accuracy Component
Tapping Speed Test	Motor Function

Protocol

Each subject arrived at the Hypo-Hyperbaric Chamber Facility and ingested either 100 mg/kg body weight of either tyrosine or a placebo in capsule form. They then completed the "How Are You" sheet and the pre-exposure briefing was conducted by the

researcher. All questions were answered prior to the subject(s) being putting on the hood and being connected to the gas supply. After putting on the hood, the subjects were left alone in the confined space and monitored from a nearby position inside the chamber by the investigator. For all altitude exposures, all personnel inside the chamber breathed supplemental oxygen for 30 minutes prior to ascent. No pre-breathing was performed for ground level exposures. Upon reaching 21,000 feet (or after a short period of time on the ground), testing was begun and the individual tests were spaced out over the three (3) hour exposure with periods of inactivity between. Upon completion of testing, the hood assembly was removed and a post-exposure briefing was conducted and the subject(s) left the chamber facility. Figure 4 represents a generic exposure timeline that was used for altitude exposures. Ground level exposures followed the same timeline, but without the 30 minutes of denitrogenation.

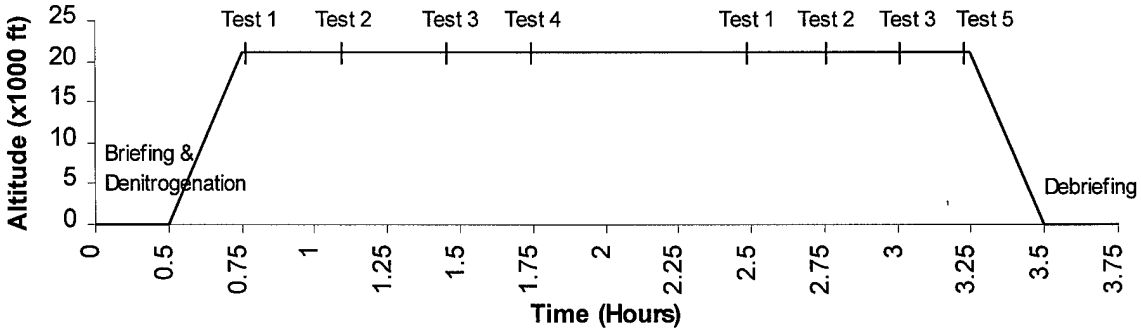

Figure 4. Exposure Timeline

Statistical Analysis

Results from the tests conducted during each exposure were compiled and analyzed using statistical measures. Tests that were performed twice during each

exposure were averaged and the average scores were analyzed. The Scanning Visual Vigilance Task and PVT had dedicated analysis software that was used to compile and group their data according to the exposure profiles. The compiled data were then analyzed using MINITAB statistical software.

Psychomotor Vigilance Task: The data were reduced by existing, custom software into the separate exposure conditions and then imported into Microsoft Excel and the mean reaction times and the number of lapses (reaction time slower than 500 msec) were calculated. The mean reaction times and the number of lapses were then analyzed.

Code Substitution Task: The data recorded by the task include the number of correct responses, percent correct, and the mean reaction time. The mean response time and the percent correct were analyzed.

Pattern Matching Task: The data recorded from the task include the number of correct responses, percent correct, and the mean reaction time. The mean response time and the percent correct were analyzed.

Simultaneity Task: The mean of the shortest intervals perceived were recorded and analyzed.

Tapping Task: The number from the second of the three trials was used for analysis.

Scanning Visual Vigilance Task: The task was run for 30 minutes and generated 150 stimuli for each trial. The number of misses, the number of false positives, and the mean reaction time were used for analyzed.

Profile of Mood States: The individual scores from each of the 6 areas were added together, with the Vigilance score being negatively weighted, to obtain a Total Mood Disturbance (TMD) score for each subject. The mean TMD scores for each exposure profile were analyzed.

Functional Acuity Contrast Test: The last correct value for each row was recorded and the scores for the left and right eye averaged. The mean value for each row was analyzed.

CHAPTER IV

RESULTS

All subjects started and completed all four exposure profiles. Results of each test were compiled for each exposure profile and the means were analyzed. Statistical analysis was performed using MINITAB statistical software and statistical significance was established at $\alpha = 0.05$. For each measure, a paired *t*-test was performed and the results were adjusted using the Bonferoni adjustment procedure by first calculating the inter-correlation matrix for the four treatment groups, then calculating the mean correlation, and finally the Bonferoni-corrected p value for significance was determined. The Pearson r correlation was transformed to a Fisher z value, averaged, and the mean Fisher z value converted back to a Pearson r value. This valued varied between all measures. Therefore, statistical significance was not identified unless the p value for each measure was below the calculated value for that particular measure.

The following comparison groups were analyzed to determine if there was any improvement in performance with the use of supplemental oxygen or supplemental tyrosine: 1) Ground-Placebo vs. Ground-Tyrosine; 2) Altitude-Placebo vs. Altitude-Tyrosine; 3) Ground-Placebo vs. Altitude-Placebo; and 4) Ground-Tyrosine vs. Altitude-Tyrosine. Only those comparisons found to be significant are reported below.

Psychomotor Vigilance Task (PVT). The variables provided by the PVT that were analyzed included the reaction time (in msec) and the number of lapses (a response time greater than 500 msec). The results are shown in Table 3 and the comparison analysis is shown in Table 4. No significant change in the number of lapses was detected and the only significant decrease in response time was observed in the Altitude-Tyrosine trial, although response time was non-significantly lower during the Ground-Tyrosine trial.

Table 3. Psychomotor Vigilance Task.

Measure	Ground-Placebo	Ground-Tyrosine	Altitude-Placebo	Altitude-Tyrosine
Response Time (msec)	217±14	211±30	214±18	202±24
Lapses (number)	0.3±0.4	0.7±12	0.7±1.0	0.3±0.4

Values are mean±SD. Lapse equals a response > 500 msec.

Table 4. Psychomotor Vigilance Task Comparison. Response Time.

Comparison	*t*	p
Altitude-Placebo vs. Altitude-Tyrosine	3.20	0.013

Scanning Visual Vigilance Task. The mean response time, number of false positives, and the number of missed stimuli were provided by the program and analyzed. The results are shown in Table 5 and the comparison analysis is shown in Table 6. No significant differences were detected in response time or number of misses, but significant reductions in the number of false positives were observed during both tyrosine trials.

Table 5. Scanning Visual Vigilance Task.

Measure	Ground-Placebo	Ground-Tyrosine	Altitude-Placebo	Altitude-Tyrosine
Response Time (sec)	0.546±0.055	0.492±0.067	0.523±0.067	0.468±0.058
False Positives (number)	5.8±2.4	2.1±1.3	4.2±1.6	1.9±1.5
Misses (number)	4.8±6.1	1.2±1.6	1.7±2.4	0.9±1.2

Values are mean±SD. Number of misses out of 150 total stimuli presented.

Table 6. Scanning Visual Vigilance Task Comparison. False Positives

Comparison	*t*	p
Ground-Placebo vs. Ground-Tyrosine	3.62	0.007
Altitude-Placebo vs. Altitude-Tyrosine	3.21	0.012*

* Not statistically significant (p value needed to be $p < 0.011$)

Profile of Mood States. The Total Mood Disturbance (TMD) score was analyzed and the only significant differences occurred with the use of tyrosine. The results are shown in Table 7 and the comparison analysis in Table 8. Statistically lower TMD scores were observed during both tyrosine trials.

Table 7. Profile of Mood States.

Measure	Ground-Placebo	Ground-Tyrosine	Altitude-Placebo	Altitude-Tyrosine
TMD Score	23. 9±26.9	6.9±20.1	14.1±13.9	2.0±13.2

Values are means ± SD.

Table 8. Profile of Mood States Comparison

Comparison	*t*	p
Ground-Placebo vs. Ground-Tyrosine	3.15	0.014
Altitude-Placebo vs. Altitude-Tyrosine	3.62	0.007

Functional Acuity Contrast Test. Visual contrast sensitivity was recorded for each of the 5 spatial frequencies and the mean score for each was analyzed. The results are shown in Table 9 and the comparison analysis is shown in Table 10. Significant increases in contrast scores were observed for many of the tyrosine trials.

Table 9. Functional Acuity Contrast Test.

Measure	Ground-Placebo	Ground-Tyrosine	Altitude-Placebo	Altitude-Tyrosine
1.5	72.5±19.8	83.8±15.6	76.1±22.2	83.2±16.2
3	112.3±26.9	122.1±27.4	114.0±29.2	133.2±24.8
6	112.2±39.6	122.4±38.0	120.1±39.8	134.8±41.3
12	58.2±32.2	66.4±32.4	59.8±26.2	72.7±29.0
18	23.5±18.0	29.5±18.8	27.6±19.0	34.6±21.6

Values are mean contrast scores ± SD. Measure is the spatial frequency (cycles per degree)

Table 10. Functional Acuity Contrast Test Comparison.
Spatial frequency in parentheses.

Comparison	*t*	p
Ground-Placebo vs. Ground Tyrosine (3)	-2.43	0.041*
Altitude-Placebo vs. Altitude-Tyrosine (3)	-3.17	0.013
Ground-Tyrosine vs. Altitude-Tyrosine (3)	-2.90	0.020
Altitude-Placebo vs. Altitude-Tyrosine (6)	-2.74	0.025
Altitude-Placebo vs. Altitude-Tyrosine (12)	-4.02	0.004

* Not statistically significant (p value needed to be $p < 0.039$)

Simultaneity Task. The shortest intervals perceived were recorded and analyzed. The results are shown in Table 11. There were no significant differences detected in any of the comparisons.

Table 11. Simultaneity Task.

Measure	Ground-Placebo	Ground-Tyrosine	Altitude-Placebo	Altitude-Tyrosine
Interval (msec)	30.6±19.5	20.5±6.7	22.8±9.9	22.6±7.6

Values are means± SD.

Code Substitution Task. The mean response time and percent correct were recorded and analyzed. The results are shown in Table 12 and the comparison analysis is shown in Table 13. No significant change in percent correct was detected among any of the comparisons. A significant decrease in response time was observed during both tyrosine trials.

Table 12. Code Substitution Task.

Measure	Ground-Placebo	Ground-Tyrosine	Altitude-Placebo	Altitude-Tyrosine
Response Time (sec)	1.86±0.43	1.74±0.49	1.70±0.51	1.28±0.60
Percent Correct (%)	96.1±3.1	95.3±17.7	94.8±3.7	97.4±2.9

Values are mean±SD.

Table 13. Code Substitution Task Comparison. Response Times

Comparison	*t*	p
Ground-Placebo vs. Ground-Tyrosine	2.69	0.027
Altitude-Placebo vs. Altitude-Tyrosine	2.83	0.022
Ground-Tyrosine vs. Altitude-Tyrosine	4.27	0.003

Tapping Speed Task. The number of successful alternations was recorded for each of the three trials and the data from the second trial were analyzed. The results are shown in Table 14 and the comparison analysis is shown in Table 15. A significant increase in tapping speed was observed with the ground level use of tyrosine. The increase in tapping speed with tyrosine at altitude was nearly significant ($p = 0.043$ instead of 0.041).

Table 14. Tapping Speed Task.

Measure	Ground-Placebo	Ground-Tyrosine	Altitude-Placebo	Altitude-Tyrosine
Number	30.7±12.0	36.9±15.9	33.0±11.1	42.4±14.9

Measure is mean number of taps ± SD.

Table 15. Tapping Speed Task Comparison.

Comparison	*t*	p
Ground-Placebo vs. Ground-Tyrosine	-3.34	0.010
Altitude-Placebo vs. Altitude-Tyrosine	-2.40	0.043*

*Not statistically significant (p value needed to be $p < 0.041$)

Pattern Matching Task. The mean response time and percent correct were recorded and analyzed. The results are shown in Table 16 and the comparison analysis is

shown in Table 17. No significant change in percent correct was detected among any of the comparisons. Significant reductions in response time were observed during both tyrosine trials.

Table 16. Pattern Matching Task.

Measure	Ground-Placebo	Ground-Tyrosine	Altitude-Placebo	Altitude-Tyrosine
Response Time (sec)	1.04±0.39	0.93±0.31	0.95±0.26	0.83±0.20
Percent Correct (%)	95.0±4.2	91.0±14.4	96.4±3.3	96.8±2.6

Values are mean±SD.

Table 17. Pattern Matching Task Comparison. Response Times

Comparison	*t*	p
Ground-Placebo vs. Ground-Tyrosine	3.28	0.011
Altitude-Placebo vs. Altitude-Tyrosine	2.67	0.028

Table 18 summarizes the results of all measures tested in the present study.

Table 18. Summary of Results.

Test Measure	Ground-Tyrosine (vs. Ground-Placebo)	Altitude-Tyrosine (vs. Altitude-Placebo)	Altitude-Placebo (vs. Ground-Placebo)	Altitude-Tyrosine (vs. Ground-Tyrosine)
PVT				
Response Time	↓	↓	N/C	N/C
Number of Lapses	N/C	N/C	N/C	N/C
SVT				
Response Time	N/C	N/C	N/C	N/C
False Positives	↓	↓	N/C	N/C
Misses	N/C	N/C	N/C	N/C
POMS				
TMD	↓	↓	N/C	N/C
FACT				
1.5	N/C	N/C	N/C	N/C
3	↓	↓	N/C	↓
6	N/C	↓	N/C	N/C
12	N/C	↓	N/C	N/C
18	N/C	N/C	N/C	N/C
Simultaneity Task				
Interval	N/C	N/C	N/C	N/C
Code Substitution				
Response Time	↓	↓	N/C	↓
Percent Correct	N/C	N/C	N/C	N/C
Pattern Matching				
Response Time	↓	↓	N/C	N/C
Percent Correct	N/C	N/C	N/C	N/C
Tapping Speed Test				
Number of Taps	↓	↓	N/C	N/C

Bolded arrow indicates statistical change; Regular arrow indicates a nearly significant change. N/C indicates no statistical change.

CHAPTER V

DISCUSSION

The use of non-pharmacological agents, such as food and herbal supplements, to improve performance and endurance is steadily increasing throughout the United States. While there is anecdotal evidence to support the use of such agents, few studies have been carried out to validate these claims. In addition, the use of such agents in combination with each other, or other medications, can have unwanted and potentially dangerous effects. In the aviation community, further restrictions are placed on what types of substances can be taken without the approval of a qualified medical practitioner. It was with these concerns and restrictions in mind that the supplemental use of the amino acid tyrosine as a safe alternative to other types of agents was proposed for use in aviation.

Numerous studies have evaluated the use of supplemental tyrosine under varying types and amounts of stress. Nearly all of these have successfully demonstrated that supplemental tyrosine reduces the performance decrements that are typically associated with stress. The results of the present study are comparable to those described by previous investigators.

Mean response time improved with tyrosine supplementation in all of the tests utilized in the present study. This improvement was present in all exposure profiles

where tyrosine was given, even when the changes were not statistically significant. Significant improvements were found with the use of tyrosine in all tests except the Scanning Visual Vigilance Task. Even though not significant, response times for the Scanning Visual Vigilance Task were still faster across all exposure profiles where tyrosine was given. Response times on the Psychomotor Vigilance Task, a task that requires sustained attention and discrete motor responses, were only significantly faster in the Altitude-Tyrosine trial (compared to the Altitude-Placebo trial). This may have been due to familiarity with the test and the test environment and would explain why the Ground-Tyrosine trial did not demonstrate a significant reduction in response time. If the improvement was due to breathing supplemental oxygen, similar improvements should have been seen in the other tasks. Response times were significantly faster on the Code Substitution Task in all exposures with tyrosine (Ground-Placebo vs. Ground-Tyrosine, $p = 0.027$; Altitude-Placebo vs. Altitude-Tyrosine, $p = 0.022$; Ground-Tyrosine vs. Altitude-Tyrosine, $p = 0.003$). It is unclear why the Ground-Tyrosine vs. Altitude-Tyrosine comparison was significant, as this is the only test where this comparison of response times was significant. Most likely this was due to learning and practice effects and not to the combination of tyrosine and supplemental oxygen. Tyrosine also improved the response times on the Pattern Matching Task (Ground-Placebo vs. Ground-Tyrosine, $p = 0.011$; Altitude-Placebo vs. Altitude-Tyrosine, $p = 0.028$). The improvements in response time suggest that tyrosine may improve motor function. This is further supported by the fact that tyrosine significantly improved performance on the Tapping Speed Task, a motor function test (Ground-Placebo vs. Ground-Tyrosine, $p = 0.010$) and

also showed a non-significant improvement during the Altitude-Tyrosine trial ($p = 0.043$).

When examining the number of lapses (response > 500 msec) on the Psychomotor Vigilance Task and the number of missed stimuli on the Scanning Visual Vigilance Task, no significant differences were detected in any of the comparisons.

The results from tests that examined visual contrast sensitivity were less conclusive with regard to the benefits of tyrosine. On the Scanning Visual Vigilance Task, where subjects detected a near-threshold stimulus, the number of false positives was reduced with the use of tyrosine (Ground-Placebo vs. Ground-Tyrosine, $p = 0.007$; Altitude-Placebo vs. Altitude-Tyrosine, $p = 0.012$). This would indicate improved contrast sensitivity by enhancing the ability to distinguish the stimulus from the background. However, results from the Functional Acuity Contrast Test are more varied and less supportive of the ability of tyrosine to improve contrast sensitivity. While scores tended to improve with the use of tyrosine, few comparisons were significant. Because spatial acuity should always be good at the lower spatial frequencies, it was not expected that changes would occur here and the results of the study support that claim. The significant improvements tended to be in the middle spatial frequencies and while at altitude. This might imply that the combination of oxygen and tyrosine improves contrast sensitivity in these spatial frequencies and could be explained by the use of tyrosine and the increased oxygenation of the eye that occurs with supplemental oxygen. If this were true, one would expect that the differences between the Ground-Tyrosine vs. Altitude-Tyrosine comparisons at the other spatial frequencies would also be significant. It is more likely that the use of tyrosine has a more significant impact on improving sustained

attention rather than improved contrast sensitivity. This fact is supported by the faster response times and tendency for fewer mistakes on the other tasks. While contrast sensitivity scores tended to improve with the use of tyrosine, few comparisons were significant. The results from this test most likely are inaccurate due to the use of a hood with a visor that is not optically correct (one that does not interfere with visual acuity). Contrast sensitivity may be improved if a proper visor (such as the visor on the full pressure suit helmet) was used. While an improvement was detected on the Scanning Visual Vigilance Task, it may be coincidental, as the Functional Acuity Contrast Test is a more sensitive contrast test. Therefore, no conclusive statements about tyrosine's effect on contrast sensitivity can be confidently made.

Cognitive performance was assessed through the use of the Code Substitution Task and the Pattern Matching Task. No significant improvements were detected in the percent correct (accuracy component of cognitive performance) for any comparison. This could be due to the lack of benefit from tyrosine or simply because the tests used to assess cognitive performance were not sensitive enough for these testing conditions. In contrast, response times (speed component of cognitive performance) for both tasks did significantly improve, as discussed above.

The use of supplemental tyrosine was shown to improve the overall mood of subjects when exposed to the various stressors present in the current study. Because there was no improvement in mood with only the use of supplemental oxygen (Ground-Placebo vs. Altitude-Placebo comparison was not significant), it can be concluded that the improvement in mood was due to the use of supplemental tyrosine and not the use of supplemental oxygen. This fact also holds true for the other measures and tasks as well.

Comparison to the Existing Literature. To my knowledge, no studies have evaluated the use of supplemental tyrosine in a normoxic hypobaric environment. It was with this in mind that the current study was designed and conducted. In comparison to the various studies that have examined tyrosine supplementation under other types of stresses and environmental conditions, the results of this study are similar.

The improvements in response time that were found with the use of supplemental tyrosine may be the result of tyrosine improving motor function. Lehnert *et. al.* and Reinstein *et. al.* showed in rats that motor deficits were alleviated and performance enhanced (46, 79, 80). Results from Deijen *et. al.* also showed an improvement in memory and tracking skill. While none of the tests used in the present study specifically examined tracking skill, the Scanning Visual Vigilance Task required subjects to continually scan the screen and watch for the appearance of the stimulus. Even though there was no significant improvement in response times, they tended to improve with tyrosine. While tyrosine did not demonstrate an improvement in the accuracy component of cognitive performance in this study, it did tend to improve response times (the speed component of cognitive performance) in nearly all tests. This would suggest that it could be beneficial in improving vigilance performance (i.e. alertness). An increase in vigilance and mood may lead to increased cognitive performance, but was simply not detected under these test conditions. The lack of any improvement in cognitive performance may also be due to the use of tests that were not sensitive enough to detect changes or because a high enough dosage of tyrosine was not used.

The results from the Profile of Mood States indicated that the use of supplemental tyrosine improved the overall mood of the subjects. The lower Total Mood Disturbance

scores when tyrosine was given resulted from decreased scores in the areas of Tension, Depression, Anger, Fatigue, and Confusion, and an increase in Vigor. Previous studies by Banderet and Lieberman reported that subjects exposed to the environmental stressors of cold and altitude displayed a decrease in adverse behavior and fewer adverse emotions with the use of tyrosine (6, 49, 87). The behaviors and emotions reported in these studies are included in the Profile of Mood States questionnaire and would suggest support for the use of tyrosine for improving mood. Gelenberg *et. al.* also reported the potential efficacy of tyrosine to treat depression (31). The results of the present study also support the use of tyrosine to improve mood.

Potential Uses for Supplemental Tyrosine. Numerous studies have demonstrated the benefits of using supplemental tyrosine in extremely stressful and challenging environments to help reduce the mental degradation that is normally associated with these conditions. This study demonstrated that the use of 100 mg/kg body weight of supplemental tyrosine is beneficial in a normoxic hypobaric environment. This shows promise for operations such as the U.S. Air Force's high altitude reconnaissance program where pilots are routinely and repeatedly exposed to extreme physiological stressors such as reduced pressure (cabin altitude of 29,500 feet), breathing dry aviator's oxygen for extended periods, possible dehydration, decreased sensory input due to the full pressure suit, immobilization due the pressure suit and confining cockpit, and long duration missions. Similar conditions exist for astronauts during extra-vehicular activities. Supplemental tyrosine may help to improve, and sustain, mental alertness and function in these operations.

Concerns with this Study. There were several areas in this study that could have benefited from closer attention.

1. A longer duration of exposure at both ground level and altitude would have been more desirable. The longer duration would have provided more of a stressor and irritant and would have allowed for more periods of inactivity between tests. The three hour duration was selected due to time constraints on the volunteers' part and it was felt that this was the minimum amount of time that would be acceptable.
2. Greater emphasis could have been placed on keeping the subjects from moving during each exposure. Although confined in a 4' x 3' space, there was still more space than in a confined cockpit.
3. The type of supplemental oxygen used, while 100% pure, contained a larger amount of moisture than the liquid oxygen used by the U.S. Air Force. While this does not affect the protocol directly, the liquid oxygen tends to dry out the respiratory system after prolong use. This dehydrates the user and act as a physiological stress.
4. The small number of subjects was a concern throughout. The large time commitment required on the part of the subjects (a total of 12 hours over 4 days) and the inability to offer any form of compensation for their time made it difficult to recruit volunteers.

Future Research. While this study provided an initial investigation of the use of supplemental tyrosine in a normoxic hypobaric environment, further study should be conducted to examine the effectiveness under different conditions.

1. The protocol should be repeated with the exposures taking place at night, preferably between midnight and 0400 hours. This would allow the researchers to test the benefits of supplemental tyrosine during the naturally occurring periods of circadian lows of both performance and tyrosine.
2. The length of exposure should be increased to provide a greater physiological stress while exposed to the test conditions.

CHAPTER VI

SUMMARY AND CONCLUSIONS

The United States Air Force's high altitude reconnaissance program imposes many stressors that are common to the aviation community, such as long hours, confining cockpit, and rotating flying schedules, on pilots in the program. In addition, there are other unique stressors, such as the full pressure suit and extreme cabin altitude, that compound the common stresses associated with flying. By flying long duration missions in such a harsh environment, pilots are routinely faced with potential decreases in alertness and performance. Pharmacological methods of dealing with the long missions and unusual duty schedules are not always practical or safe, both in the short-term and long-term. Readily available herbal supplements have not been adequately studied and their use is currently not allowed by aircrews. The addition of supplemental tyrosine to the existing "tube food" can provide a safe, effective way of dealing with performance decrements and help to reduce the challenges pilots face.

Despite numerous studies examining the use of supplemental tyrosine, little is known about its effectiveness at enhancing performance in a normoxic hypobaric environment. The results of this study do not support the initial hypothesis that breathing supplemental oxygen while exposed to a hypobaric environment enhances the beneficial effects of supplemental tyrosine. However, this study does confirm previous reports that

the use of supplemental tyrosine, even at a relatively small dose (100 mg/kg body weight), improves performance and mood in individuals who are placed in a stressful environment. While the use of supplemental tyrosine in conjunction with supplemental oxygen does not cause a further improvement in performance or mood over that which supplemental tyrosine alone provides, it may still be advantageous to the U.S. Air Force's high altitude reconnaissance program to supplement the existing in-flight ration ("tube food") with tyrosine to help improve and sustain mental performance and alertness.

The results of the present study demonstrate a potential use of supplemental tyrosine and a mechanism for further research. Extensive research remains to be done in examining the effects, benefits, and interactions of non-pharmacological agents such as food and herbal supplements. This is true for use by the general public, but especially for use by the aviation community. It is important to recognize that many substances that are deemed safe and acceptable for use on the ground may have altered effects in aviation on the physiological and psychological performance of pilots. More research in the use of such substances is important for proving their safety and effectiveness as safe, non-pharmacological methods of enhancing performance.

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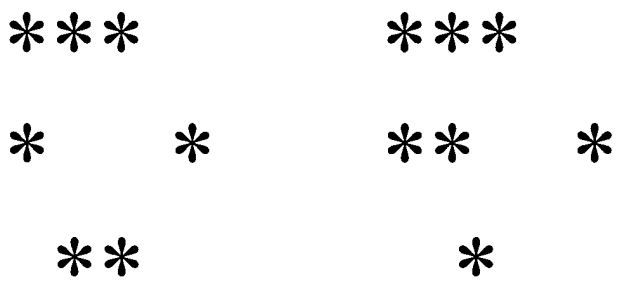
APPENDICES

APPENDIX A

PATTERN MATCHING TASK SCREEN



APPENDIX B

CODE SUBSTITUTION TASK SCREEN

S	N	P	H	U	A	T	V	B
(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)

H	V	B	S	H	U	T	S	A
(4)	(8)	(9)	(1)	(■)	( )	( )	( )	( )

P	A	V	B	P	T	B	P	U
( )	( )	( )	( )	( )	( )	( )	( )	( )

APPENDIX C

SIMULTANEITY TASK SCREEN

Stare at the fixation point

LEFT Arrow **RIGHT Arrow**

APPENDIX D

PROFILE OF MOOD STATES QUESTIONNAIRE

NAME _____ DATE _____

SEX: Male (M) Female (F)

IDENTIFICATION

Below is a list of words that describe feelings people have. Please read each one carefully. Then fill in ONE circle under the answer to the right which best describes HOW YOU HAVE BEEN FEELING DURING THE PAST WEEK INCLUDING TODAY.

The numbers refer to these phrases.

0 = Not at all
1 = A little
2 = Moderately
3 = Quite a bit
4 = Extremely

Col (C) D.P (D)

	NOT AT ALL	A LITTLE	MODERATELY	QUITE A BIT	EXTREMELY
1. Friendly	0	1	2	3	4
2. Tense	0	1	2	3	4
3. Angry	0	1	2	3	4
4. Worn out	0	1	2	3	4
5. Unhappy	0	1	2	3	4
6. Clear-headed	0	1	2	3	4
7. Lively	0	1	2	3	4
8. Confused	0	1	2	3	4
9. Sorry for things done	0	1	2	3	4
10. Shaky	0	1	2	3	4
11. Listless	0	1	2	3	4
12. Peeved	0	1	2	3	4
13. Considerate	0	1	2	3	4
14. Sad	0	1	2	3	4
15. Active	0	1	2	3	4
16. On edge	0	1	2	3	4
17. Grouchy	0	1	2	3	4
18. Blue	0	1	2	3	4
19. Energetic	0	1	2	3	4
20. Panicky	0	1	2	3	4
21. Hopeless	0	1	2	3	4
22. Relaxed	0	1	2	3	4
23. Unworthy	0	1	2	3	4
24. Spiteful	0	1	2	3	4
25. Sympathetic	0	1	2	3	4
26. Uneasy	0	1	2	3	4
27. Restless	0	1	2	3	4
28. Unable to concentrate	0	1	2	3	4
29. Fatigued	0	1	2	3	4
30. Helpful	0	1	2	3	4
31. Annoyed	0	1	2	3	4
32. Discouraged	0	1	2	3	4
33. Resentful	0	1	2	3	4
34. Nervous	0	1	2	3	4
35. Lonely	0	1	2	3	4
36. Miserable	0	1	2	3	4
37. Muddled	0	1	2	3	4
38. Cheerful	0	1	2	3	4
39. Bitter	0	1	2	3	4
40. Exhausted	0	1	2	3	4
41. Anxious	0	1	2	3	4
42. Ready to fight	0	1	2	3	4
43. Good natured	0	1	2	3	4
44. Gloomy	0	1	2	3	4
45. Desperate	0	1	2	3	4
46. Sluggish	0	1	2	3	4
47. Rebellious	0	1	2	3	4
48. Helpless	0	1	2	3	4
49. Weary	0	1	2	3	4
50. Bewildered	0	1	2	3	4
51. Alert	0	1	2	3	4
52. Deceived	0	1	2	3	4
53. Furious	0	1	2	3	4
54. Efficient	0	1	2	3	4
55. Trusting	0	1	2	3	4
56. Full of pep	0	1	2	3	4
57. Bad-tempered	0	1	2	3	4
58. Worthless	0	1	2	3	4
59. Forgetful	0	1	2	3	4
60. Carefree	0	1	2	3	4
61. Terrified	0	1	2	3	4
62. Guilty	0	1	2	3	4
63. Vigorous	0	1	2	3	4
64. Uncertain about things	0	1	2	3	4
65. Bushed	0	1	2	3	4

MAKE SURE YOU HAVE ANSWERED EVERY ITEM.

POM 021