THE VITAMIN B-6 STATUS OF PATIENTS WITH
CHRONIC OBSTRUCTIVE PULMONARY DISEASE

THESIS

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By

Anurak Bhunthurat, B.S.
Denton, Texas
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The problem of this study is to determine the vitamin B-6 status of patients who have chronic obstructive pulmonary disease (COPD). Erythrocyte aspartate transaminase assay was the method for measuring vitamin B-6 status. The vitamin B-6 status was examined in thirty subjects (ten COPD subjects and twenty control subjects).

An unpaired t-test was used to compare the vitamin B-6 status of the COPD group versus the control group. Four determinants (percentage stimulation, ratio of basal to stimulated activity, basal activity, and stimulated activity) were used to determine vitamin B-6 status in both groups of subjects. Percentage stimulation and ratio of basal to stimulated activity were not significantly different (control group versus COPD group) at the .05 level. However, two of ten COPD subjects had values for percentage stimulation that were two standard deviations above the mean, indicating a poor B-6 status. In contrast, basal activity and stimulated activity of erythrocyte aspartate transaminase were found to be significantly lower at the .05 level in the COPD group than the control group. Therefore, the COPD subjects as a group had some biochemical characteristics of a lower level of vitamin B-6 than the controls.
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CHAPTER I

INTRODUCTION

Significance of the Study

Chronic obstructive pulmonary disease (COPD) is the fifth leading cause of death in the United States (6). Data from the National Interview Survey provides estimates that there are about 7.5 million Americans with chronic bronchitis, 2.1 million with emphysema, and 6.4 million with asthma (6). In 1981 there were nearly 60,000 deaths from COPD and allied conditions, the underlying cause of 3 per cent of all deaths in the United States (6). Deaths from COPD are increasing; the age-adjusted rate rose 28 per cent between 1968 and 1978, during which time the death rate from all causes declined by 22 per cent, and rates for heart disease and cerebrovascular disease declined by 23 per cent and 37 per cent, respectively (6).

Background of the Study

Vitamin B-6 deficiency and its risks have been reported in a number of reports. It has been indicated that inadequacy may be a nutritional problem in the elderly, but it has not been established whether vitamin B-6 metabolism and requirements of the elderly differ from those of younger adults (11). Hamfelt (5) pointed out that vitamin B-6
levels in blood plasma decreased markedly with age. Driskell (4) reported that almost half of the elderly women and one-half of the elderly men, who were healthy, consumed less than 50 per cent of the RDA (Recommended Dietary Allowance) for vitamin B-6. Vir and Love (13) showed that 49 per cent of the subjects who did not take vitamin supplements had vitamin B-6 deficiency.

The risk in vitamin B-6 deficiency has also been reported among pregnant women, women taking oral contraceptive agents, and alcoholic populations. The 1980 RDA for vitamin B-6 was set at 2.0 mg (milligrams) per day for adult females and 2.2 mg per day for adult males (11). Recent evidence suggests that these levels of intake may be inadequate for a significant fraction of the population (15). Rose (12) showed that 15 to 20 per cent of the users of oral contraceptive agents had vitamin B-6 deficiency. Li (10) suggested that the incidence of vitamin B-6 deficiency in alcoholic populations probably was as high as 20 to 30 per cent. It appears that acetaldehyde (the oxidation product of ethanol) interferes with the metabolism of vitamin B-6 by promoting the degradation of pyridoxal phosphate (15). Acetaldehyde may also lower the tissue storage capacity of vitamin B-6 (15).

A poor vitamin B-6 status has also been found in various disease states, such as chronic pneumonia, bronchial asthma, and chronic bronchitis. Levkovskaia (9)
studied the pyridoxine metabolism in forty patients with chronic pneumonia, and he found that twenty-eight of the forty patients showed disorders of vitamin B-6 metabolism. He recommended the inclusion of vitamin B-6 in the therapy of patients with chronic pneumonia.

The first report describing increased excretion of tryptophan metabolites by patients with bronchial asthma was described by Knapp in 1962 (7). A tryptophan load test is one method of assessing vitamin B-6 status, and the increased concentrations of tryptophan metabolites (kynurenic acid and xanthurenic acid) in blood, sputum, or urine of patients indicate a poor vitamin B-6 status. In 1970, Warraki et al. (14) reported increased kynurenine levels in the blood of patients with bronchial asthma and chronic bronchitis. Both bronchial asthma and chronic bronchitis are in the category of COPD. In 1975, Collipp et al. (2, 3) reported increased kynurenic acid and xanthurenic acid in the urine of patients with bronchial asthma.

In 1984, Korzon et al. (8) reported the disturbances of tryptophan conversion to nicotinic acid in children with obturative bronchitis. These observed disturbances may suggest pyridoxine deficiency in these conditions.

Studies of human subjects maintained on a diet low in vitamin B-6 have also been conducted. Yess et al. (16) studied the effects of vitamin B-6 depletion on the metabolism of tryptophan in six male subjects for a period of
fifty-five days. Results of the research indicated that the subjects, who daily consumed a diet containing only 0.16 mg of vitamin B-6, had significantly increased levels of hydroxykynurenine, acetylkynurenine, kynurenine, kynurenic acid, and xanthurenic acid in the urine. Cheslock and McCully (1) conducted the study of the response of subjects to a diet low in vitamin B-6 in a 52-day experiment. The research findings showed that blood content of vitamin B-6 dropped to zero within four weeks and remained there until a supplement of pyridoxine hydrochloride was given. The researchers suggested that tissue concentration of vitamin B-6 may be expected to decrease in human beings with an inadequate intake of the vitamin, as has been shown to occur in the tissue of rats.

Statement of the Problem

This research proposed to measure and evaluate the vitamin B-6 status of patients with COPD. The vitamin B-6 status of COPD patients was compared with a control group of patients.

Hypothesis

The hypothesis that COPD patients had a significantly lower level of vitamin B-6 in red blood cell (as measured by EAST assay) when compared to a control group not suffering from chronic obstructive pulmonary disease was tested.
Delimitations

The study group included individuals with emphysema and chronic bronchitis. The control group consisted of age-matched individuals who were free from lung diseases.

The study included only subjects who had not taken vitamin B-6 and multivitamin supplements within the last two weeks.

Assumption

The assumption was that erythrocyte aspartate transaminase activity was measured properly and that this assay could help determine vitamin B-6 status.

Definitions

Asthma--a paroxysmal dyspnea, accompanied by wheezing, caused by a spasm of the bronchial tubes or by swelling of their mucous membrane.

Bronchiectasis--a chronic dilatation of a bronchus or bronchi, with a secondary infection that usually involves the lower portion of the lungs.

Bronchitis--an inflammation of mucous membrane of the bronchial tubes.

Cor Pulmonale--Hypertrophy or failure of right ventricle resulting from disorders of the lungs, pulmonary vessels, or chest wall.

Emphysema--a chronic pulmonary disease characterized by increase beyond the normal in size of air spaces distal
to the terminal bronchiole with destructive changes in their walls.

**Pyridoxine**—a group of substances, including pyridoxal and pyridoxamine, which are forms of vitamin B-6.

**Abbreviations**

ALT—Alanine Transaminase.
AST—Aspartate Transaminase.
BMR—Basal Metabolic Rate.
COPD—Chronic Obstructive Pulmonary Disease.
EEG—Electroencephalogram.
GOT—Glutamate Oxaloacetate Transaminase.
GPT—Glutamate Pyruvate Transaminase.
PLP—Pyridoxal-5-phosphate.
RDA—Recommended Dietary Allowance.
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CHAPTER II

LITERATURE REVIEW

Early chronic obstructive pulmonary disease (COPD) is characterized by an irreversible increase in small airway resistance that markedly reduces expiratory flow (11). After the administration of bronchodilators, flow generally increases only slightly. The pressure-volume characteristics of the lung are usually normal (11). Severe chronic obstructive pulmonary disease is characterized not only by an increased small airway resistance but also by an increased large airway resistance. Pathologically, severe COPD is characterized by the presence of chronic bronchitis and emphysema. Chronic bronchitis results in poorly reversible large and small airway obstruction (11).

Types of COPD

COPD is a term used to describe a variety of obstructive lung diseases, such as chronic bronchitis, emphysema, asthma, bronchiectasis, and combinations of these (23). Openbrier (23) suggested that it was more useful to separate COPD patients into subgroups, such as emphysema and chronic bronchitis, since these two diseases differed physiologically and clinically. "Pink puffer" and "blue bloater" have been used to describe patients with emphysema.
and chronic bronchitis, respectively (23). Weight loss is frequently found in the "pink puffer". In contrast, the "blue bloater" is usually overweight. These two diseases routinely coexist and share the physical alteration of chronic irreversible airway obstruction, resulting in an increased resistance to airflow through portions of the tracheobronchial tree (13).

Emphysema is a disease in which there is enlargement of the air spaces distal to the terminal bronchioles, with destruction of the interalveolar septa. Emphysema may be present alone or may be found with chronic bronchitis or, rarely, with alpha$_1$-antitrypsin deficiency (31). In the absence of antitrypsin in the serum, the proteolytic trypsin enzymes attack the lung parenchyma, and severe emphysema, especially in the lower zones, develops (31).

Chronic bronchitis is characterized by the production of excessive mucus from an inflamed bronchial mucosa, with cough productive of sputum (31). The mucous glands in the large bronchi are hypertrophied; the small airways are narrowed and inflamed (31).

Dietary Intake of COPD Patients

Two studies (9, 33) have shown that COPD patients do not have an adequate dietary intake. Driver and LeBrun (9) reported that, of twenty-six medical patients with respiratory failure necessitating ventilatory support for at least six days, the average daily vitamin, protein, and
caloric intake was inadequate in almost all cases. The average caloric intake was 1,050 calories per day. The average calculated BMR (Basal Metabolic Rate) was 1,440 calories per day. The caloric intake was equal to or exceeded the BMR in only five cases. Excluding these five, the average caloric intake was only 810 calories per day. The average daily protein intake was 26 g (grams). Only five patients received more than 40 g of protein per day. Six received no protein whatsoever. Wilson et al. (33) showed that in patients with pulmonary emphysema, shortness of breath might limit the actual quantities of food consumed. The researchers also suggested that upper abdominal distress or pain, especially after eating, and loss of appetite and weight were common symptoms of patients with pulmonary emphysema. Since COPD patients tend to have a low dietary intake, they tend to have a low intake of vitamin B-6.

Nutritional Status of COPD Patients

The nutritional status of patients with COPD has been reported. When compared to a control group, the COPD group has a significantly lower percentage of ideal body weight, triceps skinfold thickness, arm muscle circumference, transferrin and retinol-binding protein levels, creatinine height index, lymphocyte count, and vitamin E levels (4, 10, 14, 23). Albumin and prealbumin, carotene, vitamin A, thiamin,
riboflavin, vitamin C, copper, magnesium and zinc levels in the blood were also measured, but these nutrients were found to be at normal concentrations (4, 19). Vitamin B-12 levels were significantly higher in the COPD (10). Vitamin B-6 levels in the patients with COPD remain controversial. Four studies (6, 17, 28, 32) found lower levels of vitamin B-6 in the COPD group, but two studies (10, 12) found no significant differences in vitamin B-6 levels between the COPD group and the control group.

A poor vitamin B-6 status has been found in those COPD patients with bronchial asthma and with chronic bronchitis (6, 17, 28, 32). Reynolds et al. (28) reported that plasma and erythrocyte pyridoxal phosphate concentrations were significantly lower in fifteen adults with bronchial asthma than in sixteen controls. Oral supplementation of seven asthmatics with 50 mg of pyridoxine hydrochloride twice daily failed to produce a sustained elevation of pyridoxal phosphate concentrations either in plasma or in erythrocytes. The reasons for the failure of a sustained elevation in plasma and erythrocyte pyridoxal phosphate concentrations were unknown. Collipp et al. (6) reported that seventeen of twenty children with severe asthma had an abnormal increase in urinary kynurenic acid and xanthurenic acid following an oral tryptophan administration. Warraki et al. (32) reported that sixty-six patients with bronchial asthma and chronic bronchitis had higher serum kynurenine levels
than the controls. Korzon et al. (17) showed that fourteen children with obturative bronchitis had the significantly greater excretions of kynurenic acid and xanthurenic acid, compared to the controls.

Longitudinal studies have shown that COPD patients who lose weight have an increased incidence of acute respiratory failure, cor pulmonale, and high mortality (14, 23). Rogers et al. (29) have pointed out that patients with high degree of airflow obstruction tend to weigh less than those with less severe obstruction. The researchers reported that COPD patients who had lost an excessive amount of weight had a shorter survival than those whose weight was stable (29). Hunter et al. (14) reported that nutritional depletion was very prevalent in hospitalized patients diagnosed as having COPD. Openbrier et al. (23) indicated that protein calorie malnutrition was prevalent in patients with emphysema. Driver et al. (10) showed that COPD patients who developed acute respiratory failure tended to be more malnourished than those who did not develop this complication. Several studies have revealed evidence that, with advanced COPD, the patients are most often faced with one or more complications. They also have an increased susceptibility to infection. When weight loss and infection occur concomitantly, nutritional status is negatively compromised. If the effect is severe enough, protein calorie malnutrition will result (14).
Vitamin B-6 in Amino Acid Metabolism

Vitamin B-6 is a collective term for three naturally occurring pyridines—pyridoxine, pyridoxal, and pyridoxamine—that are metabolically and functionally interrelated (22). Pyridoxal phosphate (PLP) and pyridoxamine phosphate are the active forms of vitamin B-6, and they also function as coenzymes for many enzymes involved in amino acid metabolism—transamination for example (22).

Transamination is a reaction in which an amino group of an alpha-amino acid is reversibly transferred to the alpha-carbon atom of an alpha-keto acid (18). In such a reaction, catalyzed by enzymes called transaminase or aminotransferase, the tightly bound pyridoxal phosphate serves as a transient intermediate carrier of the amino group from its donor, the alpha-amino acid, to the amino-group acceptor, the alpha-keto acid. In the catalytic cycle of transaminases, the amino-group of the incoming alpha-amino acid substrate is transferred to the enzyme-bound pyridoxal phosphate. The resulting amino derivative of the coenzyme, pyridoxamine phosphate, now donates its amino group to the second substrate, the alpha-keto acid, and the coenzyme reverts to its pyridoxal phosphate form. A transamination reaction is shown in Figure 1. The reaction is written in two steps, with the transaminase and its prosthetic group designed by the two forms $E-\phi-C-H$ (pyridoxamine phosphate) and $E-\phi-C-H_{\text{NH}_2}$ (pyridoxal phosphate).
Generally, estimates of vitamin B-6 requirement in man have been based on the production or cure of clinical signs and more often on biochemical parameters (22). The biochemical parameters include plasma level of pyridoxal phosphate, the activity of serum and red blood cell B-6 enzymes, the excretion of tryptophan metabolites after a tryptophan load test, and the excretion of vitamin B-6 or its metabolite, 4-pyridoxic acid (22).

The B-6 requirement of man is increased when high-protein diets are consumed (19, 21, 22). A ratio of 0.2 mg of vitamin B-6 per gram of protein eaten has been suggested.
as a basis for calculating the vitamin B-6 allowance (22). Therefore, a daily dietary allowance of 2.2 mg of vitamin B-6 is recommended for adult males, 2.0 mg for adult females, 1.24 ± 0.70 mg for children and adolescents, 0.3 to 0.6 mg mg for infants, and an additional allowance of 0.5 mg and 0.6 mg for lactation and pregnancy respectively (22).

The use of oral steroid contraceptives may be accompanied by increased urinary excretion of tryptophan metabolites, especially after a tryptophan load test, increased stimulation of AST (in-vitro), depression, hypertriglyceridemia, and the symptom of malaise (1, 25). Pyridoxal phosphate concentrations fall in women taking oral contraceptive agents, but the decrease is temporary (20). Whether the use of oral contraceptive produces a true vitamin B-6 deficiency remains controversial. However, 15 to 20 per cent of the users of oral contraceptive agents have evidence of a vitamin deficiency (22). Nevertheless, recent studies using a depletion-repletion technique and a variety of biochemical indices indicate that the vitamin B-6 requirement for most oral contraceptive users is approximately the same as that for the non-users (8). The current evidence thus does not justify the routine supplementation of the dietary vitamin B-6 with pyridoxine for oral contraceptive users (22).
Transaminase Activity in Human Blood

Enzymes catalyzing different transamination reactions are found widely distributed in animal tissues and have shown to change in activity in some tissues during diseases (15). The two transaminases found most active in animal tissues are: (1) Aspartate transaminase (AST) or glutamic-oxaloacetic transaminase (GOT)

\[
\text{Aspartate} + \alpha\text{-ketoglutarte} \rightarrow \text{glutamate} + \text{oxaloacetate}
\]

(2) Alanine transaminase (ALT) or glutamic-pyruvic transaminase (GPT)

\[
\text{Alanine} + \alpha\text{-ketoglutarate} \rightarrow \text{glutamate} + \text{pyruvate}
\]

The effects of temperature and storage time of hemolysates on transaminase activity have been reported. Bayoumi and Rosalki (3) reported that storage of hemolysates at \(-18^\circ\mathrm{C}\) for two weeks resulted in a 17 per cent loss without PLP, and an 8 per cent loss with PLP. The researchers also reported that percentage stimulation of AST by in-vitro coenzyme addition showed minimal change after two weeks of storage at \(-18^\circ\mathrm{C}\) (3). Rose et al. (30) found that AST was stable for three to four weeks at \(-20^\circ\mathrm{C}\). Karmen et al. (15) reported that there was no change in transaminase activity with time in serum samples stored from ten minutes to ninety-six hours at room temperature, or for periods of from one hour to two weeks in the refrigerator (0-5°C). The serum transaminase activity was not changed either by freezing or lyophilization (15). No change in activity
was noted in sera subjected to 50°C for twenty-five minutes (15). Sera heated to 100°C for ten minutes were found to have lost 90 per cent of the original transaminase activity (15). No difference could be detected between transaminase activity in serum and plasma from the same donors by using oxalate, citrate, or heparin as an anticoagulant (15). Serum transaminase activity has been found to be maximal between pH 7.0 and 8.0 (15).

**Blood Cell Transaminase Activity in Normal Human**

The normal ranges of AST activity in human blood have been reported. Koj et al. (16), in a study with seven normal human subjects, reported values of 0.4 to 1.3x10^-8 uM (micromolarity) of oxaloacetate per erythrocyte per hour and 22 to 45x10^-8 uM oxaloacetate per leukocyte per hour. Wroblewski (35) reported normal serum AST activity to be 0.4 to 1.4 uM glutamate per ml per hour. Raica and Sauberlich (26) found normal ranges of AST activity to be 0.5 to 1.4x10^-8 uM per erythrocyte per hour, 21 to 38x10^-8 per leukocyte per hour, and 0.8 to 1.8 uM per ml plasma per hour. Karmen et al. (15) reported that serum AST activity varied from 0.41 to 1.36 umole (micromole) per ml per hour with a mean activity of 0.622 ± 0.191 standard deviation. Serum ALT activity was found to be between 0.21 and 1.01 umole per ml per hour with a mean value of 0.525 ± 0.146 (15). The AST activity found in hemolysates ranged from 5.0 to 8.7
umoles per ml per hour with a mean value of 6.86±0.78 while the ALT activity in hemolysates varied from 1.6 to 3.3 umoles per ml per hour with a mean value of 2.48±0.36 (15).

It has been noted that in no instance is transaminase activity absent in sera of the normal human tested or in any sera of hospitalized patients with various disease states tested (15). Karmen et al. (15) measured the transaminase activity of hospital patients, and found increased activity in the sera of one patient with lymphomatous disease, one with extensive rhabdomyosarcoma, two with arteriosclerotic heart disease and recent myocardial infarction. Serum AST activity and serum ALT activity were seen to vary in most cases of marked departure from the normal range. Somewhat greater variation was found in the transaminase activity of the hemolysates from the same patients.

Blood Cell Transaminase Activity in Human Vitamin B-6 Deficiency

There is evidence that response in blood transaminase activities in human vitamin B-6 deficiency is not as great as in rats (26). Babcock et al. (2) concluded from their studies with human subjects on a diet low in vitamin B-6 that due to the wide normal range of transaminase activity, a single determination, even at the height of deficiency, was not adequate to assess the degree of deficiency. The researchers suggested that the increase in transaminase
activity on repletion with 5 to 10 mg of vitamin B-6 per day was a good indicator of deficiency (2). It has also been reported that human subjects fed a vitamin B-6-deficient formula diet for three weeks show a slight but insignificant decrease in serum AST activity (26). On the other hand, Cheslock and McCully (5) showed in adults on a diet containing 0.4 mg vitamin B-6 per day that blood vitamin B-6 was not detectable after four weeks. Ranke et al. (27) found that aged human subjects (seventy-two years of age) had less serum transaminase activity than young persons (twenty to thirty years of age). In-vitro serum transaminase activity was stimulated by pyridoxal phosphate to a greater degree in the aged group than in the young group (27). Supplementation of the aged group with 15 mg daily vitamin B-6 for three weeks increased their transaminase activity to the same level as that in the young group (27).

Effect of Vitamin B-6 Deficiency on Human Body

Evidence has been reported that dietary deficiency of vitamin B-6 may result in numerous effects. These include impairment of both humoral and cellular immunity, convulsions in infants, hypochromic microcytic anemia, kidney stones, seborrheic dermatitis, glossitis, angular stomatitis, abnormal EEG (electroencephalogram), and hyperirritability (24, 34). An impairment in immunity has been studied in B-6-deficient animals, the majority of which show
compromised antibody production after exposure to a variety of antigens (34). Cell-mediated immune response is also affected, as indicated by the reduction in skin reactions to appropriate vaccines (delayed hypersensitivity) and the delayed rejection of foreign tissue grafts (34).

Other effects of vitamin B-6 deficiency on human subjects have also been observed. In infants, dietary deprivation of vitamin B-6 may result in epileptiform convulsions, loss of weight, abdominal distress, vomiting, and hyperirritability (24). In adults, vitamin B-6 deficiency may cause depression, confusion, and EEG abnormalities, followed by convulsions (22). The administration of the antagonist, deoxypyridoxine, to subjects receiving diets low in vitamin B-6 resulted in seborrheic dermatitis, glossitis, stomatitis, and cheilosis that responded to pyridoxine but did not respond to thiamin, riboflavin, or niacin (22).

Assessment of Vitamin B-6 Status

Several biochemical assessments of vitamin B-6 status have been used, such as measurement of erythrocyte transaminase activity with pyridoxal phosphate stimulation, measurement of pyridoxal phosphate in blood using enzymatic methods, and the tryptophan load test (34). The tryptophan load test is the usual method used for detection of pyridoxine deficiency (6). One pathway in tryptophane metabolism depends on its conversion to nicotinic acid via
kynurenine and hydroxykynurenine (6). These two reactions are catalyzed by a single enzyme, kynurenase (kynureninase), which is pyridoxine dependent and is very sensitive to variations on pyridoxal concentration (6, 7). In the early state of the deficiency, the activity of kynurenine is decreased leading to increased urinary excretion of kynurenine and 3-OH-kynurenine with continued normal excretion of xanthurenic acid (7). In the later state, the kynurenine becomes blocked further, and xanthurenic acid as well as kynurenine and 3-OH-kynurenine are excreted in excess (7). Kynurenine and 3-OH-kynurenine must undergo transamination before cyclization occurs to yield xanthurenic acid (7). The metabolism of tryptophan is shown in Figure 2 (6, 7). Asterisks indicate reactions involving pyridoxal phosphate.

Fig. 2--Metabolism of tryptophan, and * represents reactions involving pyridoxal phosphate.
Kynurenine-ketoglutarate transaminase which catalyzes the tryptophan conversion reaction is inhibited when the deficiency of vitamin B-6 is severe. And under these conditions, excretion of xanthurenic acid is markedly impaired, while the levels of kynurenine and 3-OH-kynurenine in urine continue to be high (7). It may be that this mechanism provides an explanation for the lack of xanthurenic acid excretion with the tryptophan load test that has been found in cases of vitamin B-6 deficiency. In this case, it has been suggested that the tissue levels of pyridoxal phosphate may be so low that there is marked inhibition of the activity of many enzymes which require pyridoxal phosphate as a cofactor (7).

The tryptophan load test is administered using wide range of doses of L-tryptophan (maximum 5 g), and D-L-tryptophan (maximum 10 g), with urine specimens being collected at intervals varying from four to twenty-four hours after the amino acid has been administered (7). L-tryptophan is recommended because the D form yields lower concentrations of excreted metabolites compared to the L form, and because D-L-tryptophan and D-tryptophan result in the abnormal production of large quantities of D-kynurenine (7).

There is evidence that COPD patients have a poor B-6 status. Some investigations (9, 33) have shown that they do not have an adequate food intake, in general. Their nutritional status such as ideal body weight, triceps
skinfold thickness, arm muscle circumferences, lymphocyte count, vitamin E, and vitamin B₆ tends to be significantly lower in the COPD group than the control group (4, 6, 10, 13, 14, 23, 28, 32).
CHAPTER BIBLIOGRAPHY


CHAPTER III

METHODOLOGY

Selection of Subjects

Subjects used in the study were selected from patients of the Texas College of Osteopathic Medicine in Fort Worth and the Wadley Blood Center in Dallas, Texas. Thirty subjects were used: ten were COPD patients; twenty were healthy individuals. The ten COPD patients included emphysema and chronic bronchitis patients, and they were considered as an experimental group. Patients in this group were accepted to Texas College of Osteopathic Medicine (TCOM) if they fulfilled the criteria for COPD on a previous admission. The twenty normal individuals (age-matched to the COPD patients) were people who: were not hospitalized, were free from COPD, and did not take vitamin B-6 and multivitamin supplements. These twenty normal individuals were considered as a control group. Control samples were obtained from TCOM and the Wadley Blood Center in Dallas, Texas. The vitamin B-6 status for each group was then compared.

Collection of Information on Nutritional Status

Information on nutritional status such as body weight, height, blood albumin level, and whether they took vitamin supplements, was obtained from medical records and from TCOM.
personnel who worked on the study. Other information such as smoking history, and nutritional status was obtained from the medical records.

Preparation of Blood Samples

Blood samples were collected with EDTA (ethylenediamine-tetraacetic acid) as an anticoagulant. Blood was centrifuged at 3,000 rpm (rounds per minute) for 5 minutes, and plasma and white blood cells were removed. The red blood cells were washed three times with ice-cold saline solution (9 grams of NaCl per liter) and were dispensed into 0.2 ml (milliliter) aliquots. The red blood cells were stored in the frozen state at -18°C from one to seven days. The 0.2 ml aliquots were then hemolyzed by adding 3.8 ml of distilled water and were ready for the EAST assay.

Erythrocyte Aspartate Transaminase Assay

The methodology used to determine vitamin B-6 status was erythrocyte aspartate transaminase assay, as described by Bayoumi and Rosalki (1). Measurement of erythrocyte aspartate transaminase (EAST; formerly known as EGOT) activity stimulated by in-vitro addition of pyridoxal-5-phosphate to the assay reaction provides a satisfactory indication of vitamin B-6 status since EAST activity is decreased by B-6 deprivation (2, 3). A large increase in percentage stimulation indicates a poor B-6 status. However, the in-vitro addition of pyridoxal-5-phosphate, usually in amounts of
50-100 ug (micrograms) per reaction vessel, stimulates transaminase activities significantly in erythrocytes obtained from normal individuals (2, 3). Considerable variation in EAST is seen between "normal" individuals, with or without the addition of pyridoxal-5-phosphate. The amount of stimulation varies according to the method used, but generally, with normal erythrocytes, the EAST activity is seldom stimulated more than 50 per cent (2, 3). In order to overcome some of the differences in methods of measurement and in erythrocyte transaminase activities between normal healthy individuals, the use of an erythrocyte transaminase index has been suggested (2, 3):

\[
\text{EAST index} = \frac{\text{EAST} + \text{pyridoxal-5-phosphate}}{\text{EAST} + \text{pyridoxal-5-phosphate}}
\]

Based on the report of Bayoumi and Rosalki (1) on erythrocyte transaminase activities, "normal" individuals will probably show percentage stimulation of less than mean+2SD. Higher percentage stimulation indicates lower level of blood pyridoxal-5-phosphate.

**Principle**

The EAST reaction was assayed indirectly by measuring the production of oxaloacetate, as seen in the following reaction:
Aspartate + alpha-ketoglutarate $\xrightarrow{\text{EAST}}$ glutamate + oxaloacetate

Oxaloacetate $\xrightarrow{\text{MDH}}$ malate + NAD$^+$

Decreasing concentrations of NADH were measured by following decreasing absorption at 340 nm (nanometers) because NADH had an absorption peak at 340 nm. A decrease in NADH concentration indicated that oxaloacetate had been produced by an enzymatic reaction, in which pyridoxal-5-phosphate (B-6) was a coenzyme. Since EAST is very sensitive to disturbances in pyridoxine metabolism, measurement of its stimulation by additional pyridoxal-5-phosphate is a good indicator for detecting a deficiency of vitamin B-6.

**Instruments**

Instruments used in the study were:

(1) Spectrophotometer (Bausch & Lomb Spectronic 70)

(2) Incubator chamber (water bath)

**Reagents**

Reagents used were: (1) Tris-HCl buffer: (a) Tris-HCl (Sigma), 13.22 g (grams), (b) Tris-base (Sigma), 1.94 g. These two reagents were adjusted to 1 L (liter of distilled water). The pH of the buffer should be 7.4 at 25°C. It is stable for one month when frozen. (2) EAST substrate mixture; (a) L-aspartic acid, potassium salt (Sigma), 5.13 g. (b) MDH, 0.05 ml, and (c) NADH, 21.3 mg (milligrams). The substrate mixture was adjusted to 135 ml with tris-HCl buffer
and was gently mixed and dispensed in 2.7 ml aliquots. The substrate mixture should be prepared on the day of use.

(3) Alpha-ketoglutarate, sodium salt (Sigma), 0.605 g in 10 ml of tris-buffer. Pyridoxal-5-phosphate (Sigma), 0.02 g in 10 ml of tris-buffer. Pyridoxal-5-phosphate should be protected from light and prepared on the day of use.

Procedure

Procedures used were: (A) Preincubating 0.5 ml of hemolysate with 0.05 ml of pyridoxal-5-phosphate or with 0.05 ml of tris-HCl buffer for 30 minutes at 25°C. (B) Adding 0.1 ml of the preincubated hemolysate to 2.7 ml of substrate mixture (aspartate, NADH, and MDH) and incubating for 15 minutes at 25°C. (C) Starting the reaction by adding 0.1 ml of alpha-ketoglutarate and monitoring every 5 minutes at 340 nm for 15 minutes. The activities with and without pyridoxal-5-phosphate were always measured simultaneously. Solutions of 2.7 ml of tris-HCl buffer, 0.1 ml of alpha-ketoglutarate and 0.1 ml of the corresponding preincubated hemolysate served as instrument blanks. (D) Setting up two reagent blanks from which hemolysate was omitted and running them with each batch of assay: one without pyridoxal-5-phosphate (to detect spontaneous NADH oxidation) and the other with pyridoxal-5-phosphate (to correct for apotransaminase contamination of MDH).
Analysis of Data

Data derived from calculations of EAST activity of the blood samples were analyzed using an unpaired t-test. EAST activity was calculated according to the equation (1):

\[
\frac{A \times 2.9 \times (1,000)}{5} \times \frac{6.22 \times 0.1 \times 10}{10^3}
\]

One unit of EAST activity is expressed as U/g Hb (international unit per one gram of hemoglobin) or as umole/min/g Hb (micromole per minute per one gram of hemoglobin). A/5 represents the mean decrease of absorbance of NADH per minute at 340 nm during 5 minutes. The final volume (reaction volume) is 2.9 ml. The sample volume (preincubated hemolysate) is 0.1 ml. The molar absorptivity of NADH at 340 nm is 6.22 \times 10^{-3}. The final hemoglobin concentration is 10 g/L (gram per liter).

Percentage stimulation resulting from the addition of coenzyme (pyridoxal-5-phosphate) was calculated according to the equation:

\[
\frac{[EAST(P) - EAST(P)b] - [EAST(NP) - EAST(NP)b]}{[EAST(NP) - [EAST(NP)b]} \times 100
\]

EAST(P) is the EAST activity with pyridoxal-5-phosphate, and EAST(P)b is the reagent blank for EAST(P). EAST(NP) is the EAST activity without pyridoxal-5-phosphate, and EAST(NP)b is the reagent blank for EAST(NP).
The upper limit of the normal value (mean+2SD) was calculated. Higher values were considered as indicative of vitamin B-6 deficiency. Mean EAST activity values of COPD patients and the mean EAST values of age-matched controls then were compared by using an unpaired t-test.


CHAPTER IV

RESULTS AND DISCUSSION

Results

Data of enzyme erythrocyte aspartate transaminase (EAST) activity such as basal activity (activity without added co-enzyme), stimulated activity (activity with added coenzyme) and percentage stimulation for the control group are presented in Table I. Basal activity values ranged from 0.84 to 4.52 U/g Hb with a mean value of $2.72 \pm 0.86$. Stimulated activity values varied from 2.52 to 7.23 U/g Hb with a mean value of $4.94 \pm 1.12$. Percentage stimulation ranged from 38.23 to 200.00% with a mean value of 91.09. Ages and sex of the controls are also presented in Table I. Ages ranged from 30 to 80 years with an average age of 56.

Table II includes data of ages, sex, basal activity, stimulated activity and percentage stimulation for the COPD group. Ages ranged from 31 to 81 years with an average age of 56. Basal activity varied from 0.19 to 2.84 U/g Hb with a mean value of $1.90 \pm 0.85$. Stimulated activity was found to be between 1.59 and 4.43 U/g Hb with a mean value of $3.62 \pm 0.88$. Percentage stimulation ranged from 47.89 to 736.84% with a mean value of 161.29.

Table III shows comparison of percentage stimulation between control group (group A) and COPD group (group B).
**TABLE I**  
EAST ACTIVITY IN CONTROL GROUP (GROUP A)

<table>
<thead>
<tr>
<th>Subject Number</th>
<th>Group A</th>
<th>Age</th>
<th>Sex</th>
<th>Basal Activity (U/g Hb)</th>
<th>Stimulated Activity (U/g Hb)</th>
<th>Percentage Stimulation (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 A</td>
<td></td>
<td>45</td>
<td>F</td>
<td>2.24</td>
<td>5.27</td>
<td>135.27</td>
</tr>
<tr>
<td>2 A</td>
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<td>80</td>
<td>F</td>
<td>4.52</td>
<td>6.25</td>
<td>38.27</td>
</tr>
<tr>
<td>3 A</td>
<td></td>
<td>72</td>
<td>F</td>
<td>3.12</td>
<td>5.22</td>
<td>67.31</td>
</tr>
<tr>
<td>4 A</td>
<td></td>
<td>35</td>
<td>M</td>
<td>2.66</td>
<td>4.52</td>
<td>69.92</td>
</tr>
<tr>
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<td>30</td>
<td>M</td>
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<td>5.36</td>
<td>64.42</td>
</tr>
<tr>
<td>6 A</td>
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<td>36</td>
<td>F</td>
<td>2.52</td>
<td>4.57</td>
<td>81.35</td>
</tr>
<tr>
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<td>76</td>
<td>M</td>
<td>2.28</td>
<td>4.71</td>
<td>106.58</td>
</tr>
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<td>M</td>
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<td>4.62</td>
<td>76.01</td>
</tr>
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<td>66</td>
<td>M</td>
<td>2.19</td>
<td>3.96</td>
<td>80.82</td>
</tr>
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<td>58</td>
<td>M</td>
<td>2.98</td>
<td>4.43</td>
<td>48.66</td>
</tr>
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<td>11 A</td>
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<td>50</td>
<td>F</td>
<td>0.84</td>
<td>2.52</td>
<td>200.00</td>
</tr>
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<td>79</td>
<td>F</td>
<td>1.96</td>
<td>3.40</td>
<td>73.47</td>
</tr>
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<td>72</td>
<td>F</td>
<td>3.31</td>
<td>5.59</td>
<td>68.88</td>
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<td>M</td>
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<td>F</td>
<td>3.59</td>
<td>7.23</td>
<td>101.39</td>
</tr>
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<td>36</td>
<td>F</td>
<td>2.75</td>
<td>6.06</td>
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<td>M</td>
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<td>5.22</td>
<td>107.14</td>
</tr>
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<td></td>
<td>58</td>
<td>F</td>
<td>1.35</td>
<td>3.31</td>
<td>145.19</td>
</tr>
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<td>65</td>
<td>M</td>
<td>4.20</td>
<td>6.06</td>
<td>44.29</td>
</tr>
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<td>62</td>
<td>F</td>
<td>3.03</td>
<td>4.80</td>
<td>58.42</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td></td>
<td></td>
<td></td>
<td>2.72 ± 0.86</td>
<td>4.94 ± 1.12</td>
<td>91.09 ± 1.12</td>
</tr>
</tbody>
</table>
**TABLE II**

**EAST ACTIVITY IN COPD GROUP (GROUP B)**

<table>
<thead>
<tr>
<th>Subject Group B Number</th>
<th>Age</th>
<th>Sex</th>
<th>Basal Activity (U/g Hb)</th>
<th>Stimulated Activity (U/g Hb)</th>
<th>Percentage Stimulation (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 B</td>
<td>48</td>
<td>F</td>
<td>2.84</td>
<td>4.20</td>
<td>47.89</td>
</tr>
<tr>
<td>2 B</td>
<td>81</td>
<td>F</td>
<td>2.80</td>
<td>4.43</td>
<td>58.21</td>
</tr>
<tr>
<td>3 B</td>
<td>71</td>
<td>F</td>
<td>2.47</td>
<td>4.10</td>
<td>65.99</td>
</tr>
<tr>
<td>4 B</td>
<td>40</td>
<td>M</td>
<td>2.47</td>
<td>4.20</td>
<td>70.04</td>
</tr>
<tr>
<td>5 B</td>
<td>31</td>
<td>F</td>
<td>1.63</td>
<td>2.89</td>
<td>77.30</td>
</tr>
<tr>
<td>6 B</td>
<td>38</td>
<td>F</td>
<td>2.10</td>
<td>3.92</td>
<td>86.67</td>
</tr>
<tr>
<td>7 B</td>
<td>71</td>
<td>M</td>
<td>1.86</td>
<td>3.92</td>
<td>110.75</td>
</tr>
<tr>
<td>8 B</td>
<td>58</td>
<td>M</td>
<td>1.73</td>
<td>3.96</td>
<td>128.90</td>
</tr>
<tr>
<td>9 B</td>
<td>61</td>
<td>M</td>
<td>0.89</td>
<td>2.94</td>
<td>230.34</td>
</tr>
<tr>
<td>10 B</td>
<td>58</td>
<td>M</td>
<td>0.19</td>
<td>1.59</td>
<td>736.84</td>
</tr>
<tr>
<td>Mean†</td>
<td>56</td>
<td>⋮</td>
<td>1.90†</td>
<td>3.62†</td>
<td>161.29</td>
</tr>
<tr>
<td>SD</td>
<td>⋮</td>
<td>⋮</td>
<td>0.85</td>
<td>0.88</td>
<td>⋮</td>
</tr>
</tbody>
</table>

---

† Mean values are significantly different from basal activity. SD values are standard deviations.
TABLE III
COMPARISON OF PERCENTAGE STIMULATION BETWEEN
CONTROL GROUP (A) AND COPD GROUP (B)

<table>
<thead>
<tr>
<th>Subject Group A Number</th>
<th>Percentage Stimulation (%)</th>
<th>Subject Group B Number</th>
<th>Percentage Stimulation (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 A</td>
<td>135.27</td>
<td>1 B</td>
<td>47.89</td>
</tr>
<tr>
<td>2 A</td>
<td>38.27</td>
<td>2 B</td>
<td>58.21</td>
</tr>
<tr>
<td>3 A</td>
<td>67.31</td>
<td>3 B</td>
<td>65.99</td>
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<td>4 A</td>
<td>69.92</td>
<td>4 B</td>
<td>70.04</td>
</tr>
<tr>
<td>5 A</td>
<td>64.42</td>
<td>5 B</td>
<td>77.30</td>
</tr>
<tr>
<td>6 A</td>
<td>81.35</td>
<td>6 B</td>
<td>86.67</td>
</tr>
<tr>
<td>7 A</td>
<td>106.58</td>
<td>7 B</td>
<td>110.75</td>
</tr>
<tr>
<td>8 A</td>
<td>76.01</td>
<td>8 B</td>
<td>128.90</td>
</tr>
<tr>
<td>9 A</td>
<td>80.82</td>
<td>9 B</td>
<td>230.34</td>
</tr>
<tr>
<td>10 A</td>
<td>48.66</td>
<td>10 B</td>
<td>736.84</td>
</tr>
<tr>
<td>11 A</td>
<td>200.00</td>
<td></td>
<td></td>
</tr>
<tr>
<td>12 A</td>
<td>73.47</td>
<td></td>
<td></td>
</tr>
<tr>
<td>13 A</td>
<td>68.88</td>
<td></td>
<td></td>
</tr>
<tr>
<td>14 A</td>
<td>134.01</td>
<td></td>
<td></td>
</tr>
<tr>
<td>15 A</td>
<td>101.39</td>
<td></td>
<td></td>
</tr>
<tr>
<td>16 A</td>
<td>120.36</td>
<td></td>
<td></td>
</tr>
<tr>
<td>17 A</td>
<td>107.14</td>
<td></td>
<td></td>
</tr>
<tr>
<td>18 A</td>
<td>145.19</td>
<td></td>
<td></td>
</tr>
<tr>
<td>19 A</td>
<td>44.29</td>
<td></td>
<td></td>
</tr>
<tr>
<td>20 A</td>
<td>58.42</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>91.09</td>
<td></td>
<td>161.29</td>
</tr>
</tbody>
</table>
The mean values for percentage stimulation for control group and COPD group are 91.09 and 161.29, respectively. There were no significant differences at the .05 level in the mean percentage stimulation between the two groups. Subject number 10B had the highest percentage stimulation (736.84%) in the study. That is, subject number 10B had the poorest B-6 status.

Table IV shows comparison of basal activity between control group (A) and COPD group (B). The mean basal activity for COPD group (1.90±0.85) was significantly lower than that found for the control group (2.72±0.86) at the .05 level. Subject number 10B had the lowest basal activity (0.19 U/g Hb) in the study.

Table V shows comparison of stimulated activity between control group (A) and COPD group (B). Comparing stimulated activity values of the control group with COPD group revealed a significant difference at the .05 level. The mean value for stimulated activity was found to be significantly lower in the COPD group (3.62±0.88) compared to 4.94±1.12 of the control group. Subject number 10B had the lowest stimulated activity (1.59 U/g Hb) in the study.

Table VI shows comparison of ratio of basal to stimulated activity between control group (A) and COPD group (B). The mean ratio of basal to stimulated activity for COPD group (.493±.1700) was not found to be significantly lower than the control group (.543±.1026).
TABLE IV

COMPARISON OF BASAL ACTIVITY BETWEEN CONTROL GROUP (A) and COPD GROUP (B)

<table>
<thead>
<tr>
<th>Subject Group A Number</th>
<th>Basal Activity (U/g Hb)</th>
<th>Subject Group B Number</th>
<th>Basal Activity (U/g Hb)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 A</td>
<td>2.24</td>
<td>1 B</td>
<td>2.84</td>
</tr>
<tr>
<td>2 A</td>
<td>4.52</td>
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<td>3 A</td>
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<td>2.19</td>
<td>9 B</td>
<td>0.89</td>
</tr>
<tr>
<td>10 A</td>
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<td>0.19</td>
</tr>
<tr>
<td>11 A</td>
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<td>..</td>
<td>..</td>
</tr>
<tr>
<td>12 A</td>
<td>1.96</td>
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</tr>
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<td>..</td>
</tr>
<tr>
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<td>..</td>
</tr>
<tr>
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<td>1.90±</td>
</tr>
<tr>
<td>SD</td>
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<td>0.85</td>
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TABLE V

COMPARISON OF STIMULATED ACTIVITY BETWEEN CONTROL GROUP (A) AND COPD GROUP (B)

<table>
<thead>
<tr>
<th>Subject Group A Number</th>
<th>Stimulated Activity (U/g Hb)</th>
<th>Subject Group B Number</th>
<th>Stimulated Activity (U/g Hb)</th>
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<tbody>
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<td>1 A</td>
<td>5.27</td>
<td>1 B</td>
<td>4.20</td>
</tr>
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<td>2 A</td>
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<tr>
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<td>5.36</td>
<td>5 B</td>
<td>2.89</td>
</tr>
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<td>4.57</td>
<td>6 B</td>
<td>3.92</td>
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<tr>
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</tr>
<tr>
<td>19 A</td>
<td>6.06</td>
<td>..</td>
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</tr>
<tr>
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<td>..</td>
</tr>
<tr>
<td>Mean†</td>
<td>4.94†</td>
<td>..</td>
<td>3.62‡</td>
</tr>
<tr>
<td>SD</td>
<td>1.12</td>
<td>..</td>
<td>0.88</td>
</tr>
</tbody>
</table>
TABLE VI

COMPARISON OF RATIO OF BASAL TO STIMULATED ACTIVITY BETWEEN CONTROL GROUP (A) AND COPD GROUP (B)

<table>
<thead>
<tr>
<th>Subject Group A Number</th>
<th>Ratio of Basal to Stimulated Activity</th>
<th>Subject Group B Number</th>
<th>Ratio of Basal to Stimulated Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 A</td>
<td>0.4250</td>
<td>1 B</td>
<td>0.6762</td>
</tr>
<tr>
<td>2 A</td>
<td>0.7232</td>
<td>2 B</td>
<td>0.6321</td>
</tr>
<tr>
<td>3 A</td>
<td>0.5977</td>
<td>3 B</td>
<td>0.6024</td>
</tr>
<tr>
<td>4 A</td>
<td>0.5885</td>
<td>4 B</td>
<td>0.5881</td>
</tr>
<tr>
<td>5 A</td>
<td>0.6082</td>
<td>5 B</td>
<td>0.5640</td>
</tr>
<tr>
<td>6 A</td>
<td>0.5514</td>
<td>6 B</td>
<td>0.5357</td>
</tr>
<tr>
<td>7 A</td>
<td>0.4841</td>
<td>7 B</td>
<td>0.4745</td>
</tr>
<tr>
<td>8 A</td>
<td>0.5649</td>
<td>8 B</td>
<td>0.4369</td>
</tr>
<tr>
<td>9 A</td>
<td>0.5530</td>
<td>9 B</td>
<td>0.3027</td>
</tr>
<tr>
<td>10 A</td>
<td>0.6727</td>
<td>10 B</td>
<td>0.1195</td>
</tr>
<tr>
<td>11 A</td>
<td>0.3333</td>
<td></td>
<td></td>
</tr>
<tr>
<td>12 A</td>
<td>0.5765</td>
<td></td>
<td></td>
</tr>
<tr>
<td>13 A</td>
<td>0.5921</td>
<td></td>
<td></td>
</tr>
<tr>
<td>14 A</td>
<td>0.4273</td>
<td></td>
<td></td>
</tr>
<tr>
<td>15 A</td>
<td>0.4965</td>
<td></td>
<td></td>
</tr>
<tr>
<td>16 A</td>
<td>0.4538</td>
<td></td>
<td></td>
</tr>
<tr>
<td>17 A</td>
<td>0.4828</td>
<td></td>
<td></td>
</tr>
<tr>
<td>18 A</td>
<td>0.4079</td>
<td></td>
<td></td>
</tr>
<tr>
<td>19 A</td>
<td>0.6931</td>
<td></td>
<td></td>
</tr>
<tr>
<td>20 A</td>
<td>0.6313</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean± SD</td>
<td>.5432± .1026</td>
<td>.4932± .1700</td>
<td></td>
</tr>
</tbody>
</table>
Discussion

Measurement of erythrocyte transaminase activity provides a close reflection of vitamin B-6 intake, the state of deficiency and the degree of depletion of vitamin B-6 reserves—muscle phosphorylase for example (6). According to Black et al. (2) muscle phosphorylase, a vitamin B-6 reservoir, plays an important role of pyridoxal-5-phosphate as a coenzyme in metabolism. The enzyme is accumulated with high dietary intake and declined during inadequate calorie intake (2).

In this study, the upper limit of normal value (mean +2SD) for percentage stimulation was found to be 171.38%. Higher values were considered to indicate vitamin B-6 deficiency. Our reference value (171.38%) was higher than those values reported by Hoorn et al. (4) and Bayoumi and Rosalki (1) (86 per cent and 130 per cent, respectively).

Although this study showed no significant differences for percentage stimulation, two of ten COPD patients had higher values for percentage stimulation than the reference value (171.38%). Therefore, these two COPD patients were considered to be deficient in vitamin B-6. These two patients (subjects number 9B and 10B) had severe emphysema. One of the control subjects, 11A, had higher value for percentage stimulation (200.00%) than the reference value (171.38%).
Subject number 10B had the worst B-6 status of all patients in the study. His percentage stimulation was found to be 736.84%. He not only had emphysema, but also chronic bronchitis and weight loss. Subject number 9B also had a lot of complications such as emphysema, cor pulmonale and malnutrition.

Serum albumin, an index of visceral protein stores and dietary protein adequacy, was found to be below the standard value of 3.5 g/dl (grams per deciliter) in subject number 2B (3.1 g/dl), subject number 9B (2.8 g/dl), and subject number 10B (3.3 g/dl). Serum albumin levels are related to airway function and other signs of severity of disease (3).

The results of this study showed no significant differences for the ratio of basal to stimulated activity, which, reflects the degree of saturation of apoenzyme (EAST) with coenzyme (pyridoxal-5-phosphate). In contrast, both basal and stimulated activities were found to be significantly lower at the .05 level in the COPD group. The basal activity and the stimulated activity appear to be an index of levels of pyridoxal-5-phosphate and EAST, respectively (5). Therefore, these COPD subjects had lower levels of pyridoxal-5-phosphate and EAST, respectively. It can be concluded that COPD subjects had a poorer B-6 status. This conclusion was well supported by Jacobs et al. (5).
Jacobs et al. (5) found that in the early stages of vitamin B-6 deficiency lack of coenzyme led to declined transaminase activity due to diminished saturation of the apoenzyme. In later stages chronic deficiency of the coenzyme led to decreased synthesis of apoenzyme, and if this reduced in parallel with the coenzyme its degree of saturation would remain unchanged.

Jacobs et al. also indicated that AST activity was not as sensitive as ALT to the vitamin B-6 decrease due to its greater avidity for the pyridoxal-5-phosphate. They (5) reported that there was no correlation of erythrocyte AST (basal activity, stimulated activity, and ratio of basal to stimulated activity) with age. This finding ensures that a poorer B-6 status of the COPD subjects when compared to control subjects, was not resulted from increasing of their ages.

Jacobs et al. (5) showed that a decrease in erythrocyte ALT activity (basal activity and stimulated activity) with increasing age was found in both women and men. When the activity was stimulated in-vitro with pyridoxal-5-phosphate, there was an increase in most cases but the decrease in total activities was still apparent.

Pyridoxal-5-phosphate can pass through red cell membrane and the basal activity of ALT in the cell is related to the concentration of pyridoxal-5-phosphate in the plasma (5). There is a decrease in plasma pyridoxal-5-phosphate
with age (5). Therefore, it is likely that the similar decrease in the basal ALT activity with increasing age is a consequence of this condition (5).
CHAPTER BIBLIOGRAPHY


CHAPTER V

SUMMARY, CONCLUSION AND RECOMMENDATION

Summary

Individuals with chronic obstructive pulmonary disease had a lower B-6 status than an age-matched control group. Thirty subjects were included in the study. The control group was comprised of eight men and twelve women with an average age of 56. Their ages ranged from 30 to 80 years. The COPD group was comprised of five men and five women with an average age of 56. Their ages ranged from 31 to 81 years.

The study was conducted to compare the vitamin B-6 status of COPD patients with the controls. It showed no significant differences at the .05 level for percentage stimulation and the ratio of basal to stimulated activity between the COPD group and the control group. However, two of ten COPD subjects had higher values for the percentage stimulation than the reference value (171.38%). Therefore, these two COPD subjects were considered to be deficient in vitamin B-6. These two COPD subjects were emphysema patients, frequently found to be malnourished. In contrast, basal activity and stimulated activity were found to be significantly lower at the .05 level in the COPD group than in the control group. These values of activity indicated that COPD
subjects had lower levels of pyridoxal-5-phosphate (one form of vitamin B-6) and apoenzyme (EAST). Therefore, it can be concluded that COPD patients had relatively poor B-6 status.

Conclusions and Recommendations

The results of this study did partially support the hypothesis that COPD patients had a significantly lower level of vitamin B-6 in red blood cell (as measured by EAST assay) when compared to a control group not suffering from chronic obstructive pulmonary disease. Four determinants (percentage stimulation, ratio of basal to stimulated activity, basal activity, and stimulated activity) were used in the study to point out the differences in B-6 status between the two groups of subjects. Two determinants, percentage stimulation and ratio of basal to stimulated activity, were found to be insignificant at the .05 level. The other two determinants, basal activity and stimulated activity, were found to be significant at the .05 level. These results indicate that a chronic deficiency of the B-6 coenzyme led to a decreased level and decreased synthesis of aspartate transaminase. In conclusion, this study apparently showed that chronic bronchitis and emphysema patients had relatively poor B-6 status.

In order to accomplish further insight into this area, we recommend the following:
1. Using larger sample size of subjects.

2. Conducting the experiment using another method of vitamin B-6 assay in addition to the EAST.

3. Determining the dietary history of the patients. For instance, high alcohol consumption could increase the risk of vitamin B-6 deficiency.

4. Taking the basal metabolic rate (BMR) into account to see the energy need of the patients.

5. Exploring any difficulties in eating due to shortness of breath. A modified food intake could result from difficulties in eating certain kinds of food.
CHAPTER BIBLIOGRAPHY

BIBLIOGRAPHY

Books


Articles


