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STUDIES ON EXPERIMENTAL THIAMINE DEFICIENCY IN MALE WISTAR RATS

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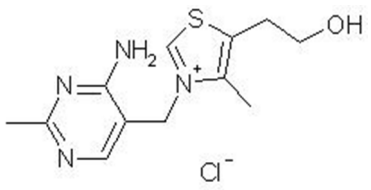
ABSTRACT

The present study investigated the Thiamin deficient in male wistar rats. Male albino rats weighing between 150-200 gm were used. They were divided into two groups and allowed to acclimatize to the new environment conditions of our laboratory for one week before use. They had free access to food and water. The standard group animals were received specified diet (Control rats) (C groups). This provides them the essential dietary requirements. The test animals received polished rice and drinking water was made available to animals all the time. The Serum glutamate oxalo acetate transminase (SGOT), Serum glutamate pyruvate transminase (SGPT), Total protein, Albumin, Serum cholesterol Serum Triglycerides Blood urea, Hemoglobin, High density lipoprotein (HDL) and rectal temperature of the rats were determined in serum, body weight and temperature recorded every alternative day. In the present study, the thiamin deficient rats showed the increased levels of SGOT, SGPT, pyruvic acid, total protein, Blood urea Hemoglobin, Serum triglycerides Serum Cholesterol and decreased body weights, HDL, Albumin and rectal temperature when compared with the normal diet received rats**.** Thiamin deficiency is due to excessive washing of rice, and low intake of fruits and vegetables.

Keywords: Thiamine, Rice, SGOT, SGPT, Body weight,

INTRODUCTION

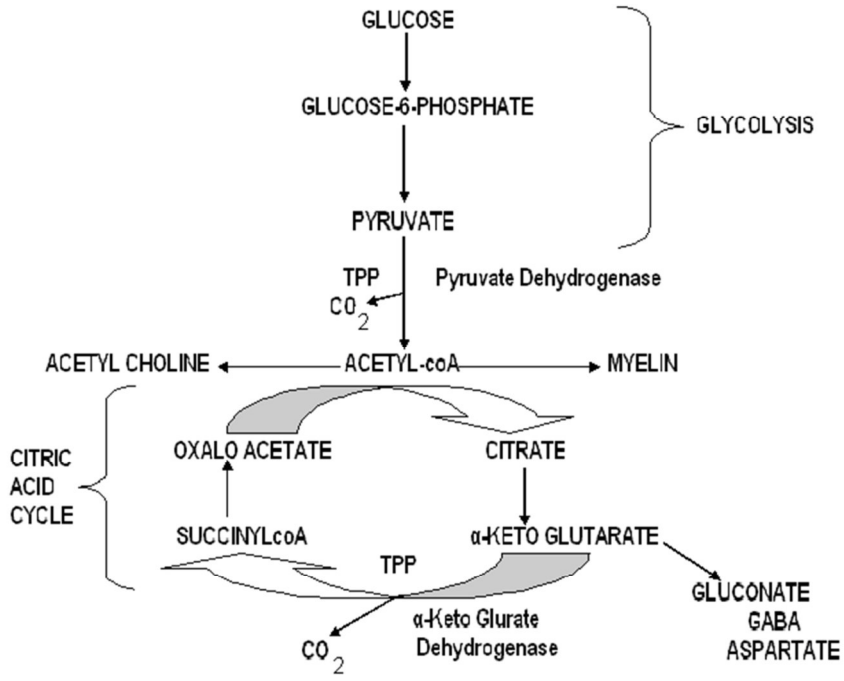
Thiamin is water-soluble B vitamin previously known as vitamin B, or aneurine. It consists of a pyrimidine ring (2, 5–dimethyl6–aminopyrimide) and a thiazolium ring (4-methyl-5-hydroxy ethyl thiazole) joined by a methylene bridge [1.]



Thiamin occurs in human body as free thiamin and as various phosphorylated forms:
Thiamin monophosphate (TMP), Thiamin triphosphate (TTP) and
Thiamin pyrophosphate (TPP), which is also known as thiamin diphosphate.

In 1911, the chemist Casimir Funk isolated a substance from rice bran extracts which was later called as thiamin or vitamin B_1. It is a water-soluble vitamin; structurally it consists of a substituted pyrimidine ring joined by a methylene bridge to a substituted thiazole ring. The free vitamin is a base. The thiazolium salts of thiamin, thiamin hydrochloride and thiamin mononitrate, are the forms of thiamin which are typically used in nutritional supplements and for food fortification. In addition to being known as vitamin B1, thiamin is known as aneurine and 3-[(4-amini-2-methyle-5-pyrimidinyl) methyl]-5-(2-hydroxyethyl)-4-methyl-thiazonium[2]. Good source of thiamine Cereals, pulses, oil seeds, nuts and yeast are good sources. It is mostly concentrated in outer layer (barn) of cereals. It is present in animal foods like pork, liver, heart, kidney, milk etc. Thiamin has been found to protect against lead induced lipid per oxidation in rat liver, kidney. Thiamin deficiency results in selective neuronal death in animal models[3]. The neuronal death is associated with increased free radical production, leading to oxidative stress, which plays an important role in brain damage associated with thiamin deficiency [4]. There is a type of anemia called thiamin-responsive megaloblastic anemia, which responds well to large doses of thiamin. It is thought that those with thiamin responsive megaloblastic anemia may actually be thiamin-deficient secondary to reduced thiamin transport and absorption and to impaired intracellular thiamin pyrophosphorylation. Thiamin deficiency is associated with cognitive and emotional changes. For example, Korsakoff's psychosis is characterized by the inability to form new memories, the poorly organized retrieval of remote memories, apathy and emotional blandness. The treatment of korsakoff's patients with thiamin often results in significant improvement in the symptoms. Marginal thiamin deficiency may also results in cognitive and emotional changes. Few studies suggest that high dose thiamin supplement action may have a positive effect on mood and even adequate status of the vitamin. The mechanism of the neurophysiologic action of thiamin is not well understood. Thiamin may play role in neurophysiology independent of its coenzyme function. Thiamin is located in nerve cell membranes and phosphorylated forms of thiamin are associated with sodium channel proteins. It is thought that phosphorylated forms of thiamin may play roles in control of sodium conductance at axonal membranes and in other neurological process [5].

Thiamin has been found to inhibit thiamin deficiency which is a common problem in alcoholics, the elderly and chronically ill. Thiamin supplementation is useful in these groups and it helps to protect against some of the metabolic imbalances caused by heavy alcohol consumption. It protects against wernicke's encephalopathy and some other forms of brain damage seen in some alcoholics, some with HIV-diseases, some with anorexia nervosa and others. It may help in with alcohol withdrawal. It is needed in those who receive total parenteral nutrition, particularly to prevent lactic acidosis (condition caused build up lactic acid in the body and lead to acidification of blood) due to thiamin deficiency. It may increase glucose tolerance and may help to prevent atherosclerosis, particularly in diabetics. It has been used in congestive heart failure and some heart diseases. It can improve mood and cognition in some [6]. The other two enzymes PDH and α-KGDH, also participate in different steps in the breakdown and conversion of glucose-6-phosphate through two consecutive chains of biochemical reactions called glycolysis and citric acid cycle. The main function of these pathways is the generation of a molecule called adenosine triphosphate (ATP), which provides energy for numerous cellular process and reactions. Proper functioning of PDH is essential for the production of the neurotransmitter acetyl choline as well as for the synthesis of a compound called myelin, which forms a sheath around the axons of many neurons ensuring the ability to conduct signals through neurons. The citric acid cycle and α-KGDH play a key role in maintaining the levels of the neurotransmitters glutamate, gamma-amino butyric acid (GABA) and aspartate, as well as in protein synthesis. Decrease in the activities of PDH and α-KGDH can result in reduced ATP synthesis leading to cell damage and even death.



MATERIALS AND METHODS

2. Rice powder

Polished rice was prepared by washing and rubbing the old stock rice for 10 min and soaked in water overnight. The water was drained off and again rice was cooked. The cooked rice was dried at 80^0c and powdered in a mill. This processing reduces the thiamin content of rice, because thiamin is richly present in outer layers of rice. To this polished rice tea powder was also mixed as it serves anti thiamin and it is added to observe the symptoms early.

3. ***Normal diet pellets***

Normal pellets diet was purchased from Sri Raghavendra Enterprises, Bangalore.

4. Animals

Male albino rats weighing between 150-200 gm were used. They were divided into two groups and allowed to acclimatize to the new environment conditions of our laboratory for one week before use. They had free access to food and water. Before start of the work the rats were marked with numbers and weighed. The standard group animals were received specified diet. This provides them the essential dietary requirements. The test animals received polished rice and drinking water was made available to animals all the time. During the test, the cages were kept clean and animals are supplied no food other than specified. The first six weeks of the test, each animal was weighed. The body weight and rectal temperature of the rats were recorded every alternative day and recorded in the tables. On the start of fourth week the following symptoms are observed.

5. Acute form (rare)

It is characterized by loss of weight, inactivity unless disturbed. These characters are developed within 30-35 days after the animal has been on the polished rice diet.

6. Chronic form (most common)

It is characterized by paralysis of the extensors of legs, neck and finally entire body. These symptoms were observed after 5-6 weeks of treatment with polished rice diet[9].

7. ANALYSIS OF BIOLOGICAL SAMPLES

7.1 Blood sample preparation

The minimum quantity of biological samples required for lab tests was obtained by giving moderate anesthesia and painless methods are followed during the entire experimental procedure. Blood samples were obtained at various intervals from the retro-orbital sinus. A measured volume of blood was quickly mixed with five volumes of ice cold 10 % (w/v) trichloroacetic acid in a test tube. Shaken vigorously for 10 sec to break up any lump formation. Centrifuged at 1000 rpm for 5 minutes and the clear supernatant were used for analysis[11].

7.2 Estimation of pyruvic acid

A sample (0.2 ml) of deproteinised blood was allowed to react with 0.1 ml of 2,4dinitrophenyl hydrazine reagent (0.1% in 2N Hcl) in a glass stoppered test tube at 37^0c. The pyruvic acid, dinitrophenyl hydrazine was extracted by shaking the mixture with 1 ml of chloroform for 30sec. After the mixture had been centrifuged for 3 min at 1500 rpm, the lower aqueous layer was removed with a fine capillary pipette and the chloroform layer was shaken for 30 sec. With 1 ml of 10% (w/v) sodium carbonate solution. The mixture was centrifuged for 5 min at 1500 rpm and 0.7 ml of carbonate extract was transferred to a comparison cell and after mixing with 2 ml of 2N sodium hydroxide, the extinction of the colour developed was measured at 520nm in a spectrophotometer within 5 min. The sodium hydroxide concentration in the final solution was 0.75N, which is optimum for the development of reddish colour of the hydrazones. For more accurate estimation, the yellow colour of the carbonate was read at 370nm or at 420nm in a micro cuvvette[11].

7.3. CHOD/POD – Phosphotungstic method

The control kit comes with Enzyme reagent, Precipitating reagent and Standard – 200mgs%. Generally in a normal man the percentages are 130-250 Total cholesterol, HDL cholesterol: Men: 30-70mgs% and in Female: 35-90mgs%, Specimen: Serum or heparinised or EDTA plasma.

7.4. Procedure for HDL (High density lipids) assay

Initial steps taken were collecting the samples into centrifuge tubes by pip petting of Serum or plasma about 0.2ml along with the precipitating reagents were mixed well and allowed to stand for 5 minutes at room temperature. Then centrifuged at 3000 rpm for 10 minutes to get a clear SNF (supernatant fluid).

7.5. Serum total protiens

Proteins are organic nitrogenous compounds of the body made of amino acids, which are linked by peptide banks. The elemental compositions of proteins are carbon, hydrogen, oxygen, nitrogen, sulphur, phosphorus, iron and other metals. Proteins from the basic structural component of the body. Serum proteins are albumin and four components of globulin – (α) alpha 1, alpha 2, beta and gama. Simple proteins are albumin and globulins. Conjugated proteins are haemoglobin, nucleo-protiens, phospho- proteins and lipoproteins. Serum protein estimation can diagnose liver disorders, nutritional deficiency of proteins, renal failure.

7.6. Estimation of total protiens

Three test tubes were taken and marked as blank (B), standard (S), test (T), then added one ml of Biuret reagent and distilled water two ml in all the three tubes. The standard 0.05 ml added only Standard test tube and serum or plasma 0.05 ml in test (T) tubes. Test tubes were mixed well and incubated at 37^0 c for 10 minutes. The optical density of standard and test were measured against blank with yellow green filter or at 555 nm.

8. ESTIMATION OF ALBUMIN

8.1. BCG (Bromocresol green) dye binding method

Three test tubes were taken and marked as blank (B), standard (S), test (T), then added one ml of Buffered dye reagent and two ml of distilled water in all three tubes. The standard 0.01 ml added only Standard test tube and serum or plasma 0.01 ml added in test (T) tubes. The test tubes are mixed well and measured immediately. The absorbance of test and standard are done against blank with a red filter or 630nm [7].

8.2. Estimation of haemoglobin

Sahil`s haemoglobinometer with a dilution tube, micro pipette, stirrer and 0.1N Hcl is used. The decinormal Hcl (0.1N Hcl) was taken in a graduated dilution tube upto mark “2” (on gms %). The dilution tube was kept in the central column. Blood samples were collected from the retro orbital sinus route. The blood was sucked in micropipette up to 20 µ mm. The blood was transferred into the dilution tube (simply touch the tip of the micropipette to the surface of Hcl, do not blow). The blood and 0.1N Hcl were stirred with a stirrer and allowed to settle for five minutes. This is stirred well after adding adding each drop of distilled water and continued till the colour of mixture matches with the standard standard colour on the lateral surface. The dilution tube is removed and the scale of haemoglobin is measured in % and gms. For estimation of SGPT, SGOT, Urea, Tri-glycerides, levels minimum quantity of blood samples were sent to Sai Apollo Diagnostic center, Kadapa, and reports were collected.

9. Student's t-test

The t- test is the common statistical procedure used in biostatics. W.S. Gosset in 1908 discovered the student t- distribution for small samples and it was then perfected by R.A. Fisher in 1926. This test is applied to assess the statistical significance if difference between two independently drawn sample means obtained two series of data with an assumption that the two means are from normal distributed populations, with no significant variations. Higher the t value greater would be the chance of significance. It is related to the total number of observations. The comparable numbers are expressed as numbers of degrees of freedom which is computed as n_1+n_2-2. The P-value indicates whether the observed difference in the means is statistically significant or not.

RESULTS

The values were mentioned in the table 1. Serum glutamate oxalo acetate transminase (SGOT), Serum glutamate pyruvate transminase (SGPT), Total protein, Albumin, Serum cholesterol Serum Triglycerides Blood urea, Hemoglobin, High density lipoprotein (HDL) and rectal temperature of the rats were determined in serum, body weight and temperature recorded every alternative day. In the present study, the thiamin deficient rats showed the increased levels of SGOT, SGPT, pyruvic acid, total protein, Blood urea Hemoglobin, Serum triglycerides Serum Cholesterol and decreased body weights, HDL, Albumin and rectal temperature when compared with normal diet received rats**.** Thiamin deficiency is due to excessive washing of rice, and low intake of fruits and vegetables. The body weight and Temperature and Pyruvic acid were mentioned figure 1, 2 &3.

Table 1

Effect of thiamin deficiency on body weights, rectal temperatures, SGPT, SGOT, total proteins, albumin, serum cholesterol, HDL cholesterol, serum triglycerides, blood urea, haemoglobin content.

Groups	Control Groups	Test Groups
Initial body weight (gms)	100 ± 1.41	100 ± 1.41
Final body weight (gms)	137 ± 0.707	90 ± 0.816
Body weight after thiamin administration	140 ± 0.7	96 ± 0.81
Initial rectal temperature (^{0}C)	37.8 ± 0.108	37.8 ± 0.707
Final rectal temperature (^{0}C)	39.4 ± 0.04	35.9 ± 0.028
SGOT IU/L	45.1 ± 1.2	247.85 ± 6.07
SGPT IU/L	65.12 ± 1.25	237.7 ± 6.05
Total proteins gm/dl	2.23 ± 0.07	6.775 ± 0.192
Albumin gm/dl	4.25 ± 1.45	3.575 ± 0.108
Serum cholesterol mg/dl	72.91 ±9.21	99 ± 1.29
HDL cholesterol mg/dl	50 ± 1.3	45 ± 0.408
Serum triglycerides mg/dl	92.5 ± 4.62	102.25 ± 1.65
Blood urea mg/dl	32.5 ± 2.87	34.8 ± 2.58
Hemoglobin content gm%	10 ± 1.08	11.65 ± 0.55

Values are MEAN ± SEM

Figure 1

Changes in body weights of rats fed on a thiamin deficient diet T groups and rats fed with normal diet C groups

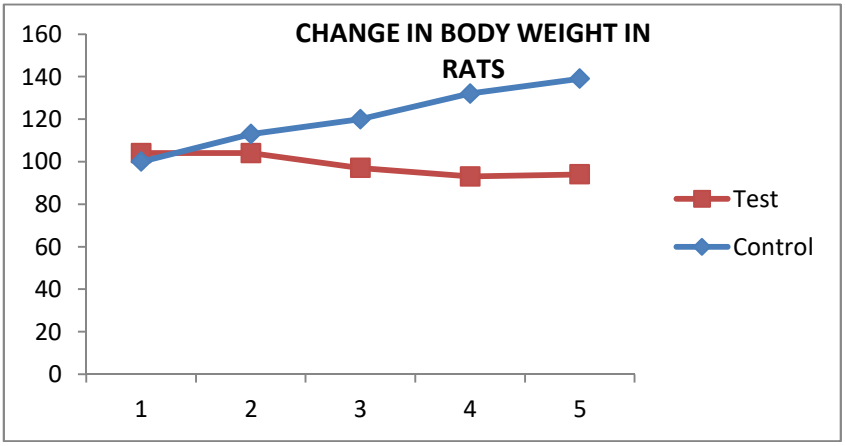


Figure 2

Changes in rectal temperature of rats fed on thiamin deficient diet T groups and rats fed with normal diet C groups

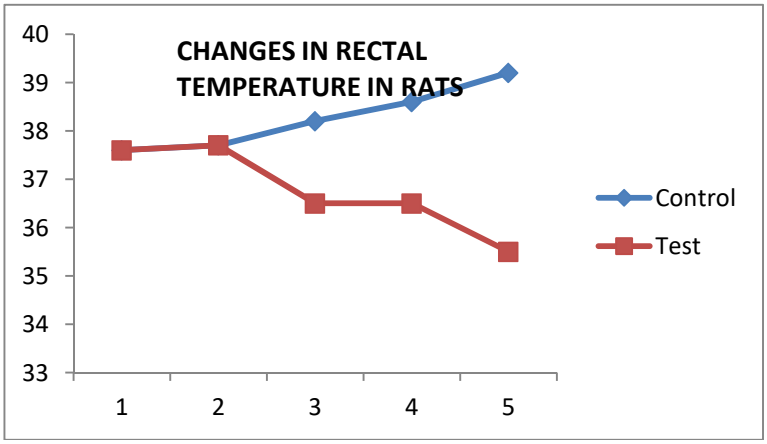
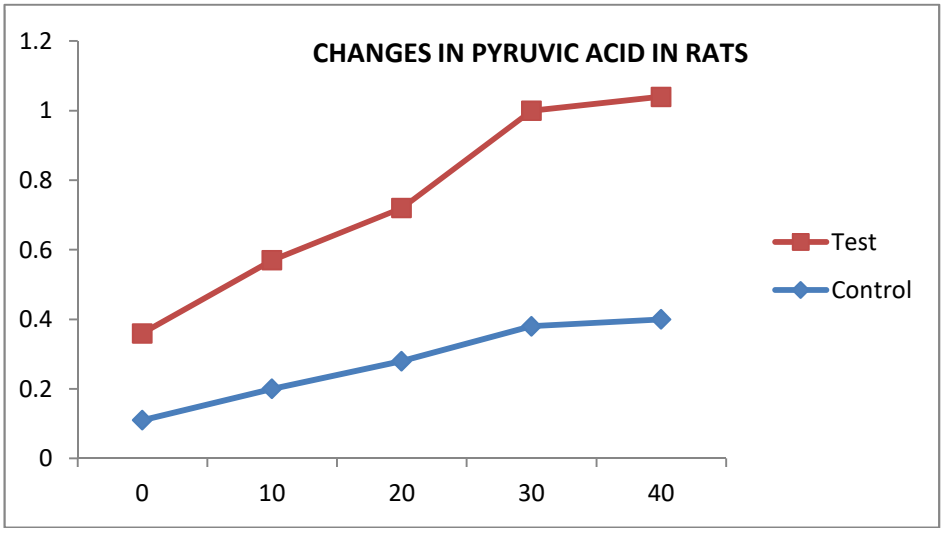


Figure 3

Changes of pyruvic acid concentration in blood of T groups of rats during the period of thiamin depletion.



DISCUSSION

Upon the experiments which were conducted on Wister rats for thiamin deficient food, has proven that when the wistar rats fed with rice which was dehusked, polished, tea powder added showing the differences. Considerations of table no: 01 and fig-1-3, it is evident that when animals fed with thiamin deficient diet began to lose weight. Anorexia was the first sign of thiamin deficiency due to reduced food intake. They were weakened and their rectal temperature fell. Then, suddenly and without much warning in the way of gross symptoms, the rats developed convulsions (polyneuritis). This symptom is also developed due to restricted feeding. From the table 1 and fig 3 the pyruvic acid concentration are steadily raised after administration of thiamin deficient diet. This may be related to the reduced metabolic rate. In the present study, it was found that there is steep raise in SGOT & SGPT levels but total proteins, albumin, serum cholesterol, HDL, serum triglycerides and blood urea values remain within the normal range. It is possible that thiamine deficiency can influence aminotransferase activites by mechanisms partially independent of food intake. For example, the accumulation of metabolites such as pyruvic acid in thiamine-deficient animals may affect aminotransferase activity. Concomitant deficiencies of other B-vitamins and the metabolic interactions of the deficiency states could also produce enzyme responses which might obscure the response to a simple pyridoxine deficiency [8].

The fall in keto acid concentrations that occurred from 20 to 40 days on the diet in experimental thiamine deficiency has been reported and its mechanism is observed. The second fall in keto acid concentrations may be related to the reduced metabolic rate, which becomes very low a few days before death[9]. In view of the considerable variation in the concentration of keto acids noted during the course of thiamine depletion, the timing of the collection of samples might account for the conflicting results for the pyruvic acid concentrations in the blood and urine in previous reports [10]. In the present experiments it was found that the second rise in total keto acid and pyruvic acid concentrations coincided with the rapid rise in concentration of both acids in the blood of thiamine deficient rats. From the known experimental studies and the clinical findings in the course of thiamine deficiency it seems that the nervous function is one of the first to be disturbed. It is known that nervous tissue has high concentrations of glutamic acid and glutamine. Therefore, it may play a significant role in the abnormal metabolism in thiamine deficiency.

CONCLUSION

In the present study, the thiamin deficient rats showed the increased levels of SGOT, SGPT, pyruvic acid and decreased body weights, rectal temperature when compared with normal diet received rats**.** Thiamin deficiency is due to excessive washing of rice, and low intake of fruits and vegetables.

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