Effect of thiamin deficiency on energy metabolites in the turkey

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The effects of thiamin deficiency on selected energy-related metabolites was investigated. A basal diet (B) was formulated to be 11% of NRC recommended level of 2 mg/kg of thiamin. Thiamin was added to this basal diet to generate the control diet (C). Twenty one-week-old female turkeys were fed either the B or C diet. On days four and five of the experiment, food intake was decreased significantly in B fed turkeys ($P < 0.05$). Plasma and brain samples were collected at this time. Brains were dissected and analyzed for ATP, ADP, uric acid, free fatty acids, glucose, and GABA. Adenosine triphosphate and the ATP/ADP ratio were decreased in the hindbrain (medulla-pons area) of thiamin deficient birds ($P < 0.01$). Uric acid was increased ($P < 0.001$) and free fatty acids were decreased ($P < 0.0005$) in the plasma of thiamin deficient birds. Based on the data, changes in ATP and ATP/ADP levels may be related to the anorectic behavior exhibited by the thiamin deficient bird.

Keywords: thiamin deficiency; turkey; brain; ATP

Introduction

Thiamin deficiency has been documented in a variety of species including pigeons,[1] chickens,[2] monkeys,[3] and rats.[4] Total brain thiamin concentration can be reduced by 50% before clinical signs of deficiency appear. Loss of thiamin leads to anorexia and neurological symptoms.[4] Brain areas with the highest thiamin turnover are the most sensitive to thiamin depletion,[5] while the most sensitive area appears to be the brainstem.[6] Cerebral glucose utilization decreases with decreasing cerebral thiamin concentration.[7]

At the cellular level, thiamin acts as a coenzyme for pyruvate dehydrogenase in pyruvate oxidation,[8] alpha ketoglutarate dehydrogenase in the Kreb's-Citric Acid-TCA cycle[9] and transketolase in the hexose monophosphate shunt.[10] Transketolase is the first enzyme affected by thiamin deficiency,[6] and it is also the last to recover.[11,12] Alpha ketoglutarate dehydrogenase activity can be decreased by 30% in dietary thiamin deficiency.[13] The effect on pyruvate dehydrogenase activity is variable according to the vehicle used to induce thiamin deficiency. Dietary thiamin deficiency affects brainstem structures primarily at early stages.[13] Antagonists, such as pyrithiamin, can cause more widespread damage than dietary deficiency at early stages.[13]

Thiamin deficiency in the rat does not affect the concentrations of adenine nucleotides in the cortex or brainstem,[14] yet decreased ATP levels are found in the lateral vestibular nucleus of pyrithiamin-treated rats.[9] The objective of this work was to determine if there are changes in energy metabolites in the brain and plasma of thiamin deficient turkeys.

Materials and methods

Twenty one-week-old female poults were randomly assigned to experimental or control groups. Birds were housed individually. A basal diet was formulated to be 11% of NRC recommended level of 2 mg/kg of thiamin and was fed to the experimental group. The control birds received a basal diet with thiamin added to meet the recommended level of 2 mg/kg.[15] Celufil, a non-nutritive bulk substance (U.S. Biochemical Corporation, Cleveland, OH) was added to the experimental diet to counter the thiamin added to the control diet. Food intake and body weight of each bird was recorded daily. When feed intake declined significantly ($P < 0.05$) for two consecutive days, all birds were decapitated into a bath of dry ice and acetone.

Heparinized blood samples were also taken. Brain tissue was stored at −80°C. Blood was spun in a re-

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frigerated centrifuge at 3500*g* for 6 minutes. Plasma was stored at −20°C.

The cerebellum and optic lobes were removed and the remaining tissue was sectioned into fore, mid, and hindbrain areas on a platform cooled by dry ice and acetone. The optic lobes were removed from the midbrain by two sagittal cuts made 4 mm on either side of the brain midline. The cerebellum was sectioned from the medulla-pons (hindbrain) by separation at the point where cerebellar striation ends (anterior to the nucleus of Edinger-Westphal). The forebrain refers to the cortical lobes which have been separated at the point nearest the cortex. By sectioning at the occulomotor nerve, the remaining tissue was split into the midbrain (anterior) and hindbrain (posterior).

All dissection was based on the pigeon atlas of Karten and Hodos.[16] Brain tissue was assayed for ATP,[17] gamma aminobutyric acid (GABA),[18] and ADP.[19,20] Glucose,[21] uric acid,[22] free fatty acids (FFA),[23] and β-hydroxybutyrate[24] were determined in plasma samples.

Data were analyzed using analysis of variance.[25]

Results

Food intake of thiamin deficient birds was decreased by the fourth and fifth days on experiment (*Figure 1*). Brain analysis revealed a decline in ATP and the ATP/ADP ratio in the hindbrain of deficient birds (*Table 1*). In all the areas analyzed, GABA and ADP concentrations were not altered in either group (*Table 1*). In the fore and midbrain, ATP and the ATP/ADP ratio were not significantly different between the two groups (*Table 1*). Plasma glucose and β-hydroxybutyrate were not changed, but plasma uric acid concentration was increased and FFAs decreased in deficient birds (*Table 2*).

Discussion

In young rats, dietary thiamin deficiency symptoms do not appear until the animals have been on experiment for 10 to 12 days.[4] In birds placed on study at one week of age, deficiency symptoms appeared after day 4 on experiment. The onset of weight loss and intake reduction is rapid and not reversible without thiamin repletion or force-feeding.[4]

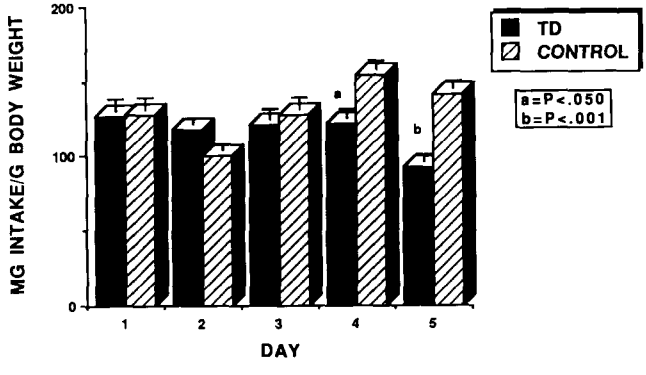

Figure 1 Food intake of thiamin deficient and control poults during the five-day study. By days four and five, thiamin deficient birds had a significantly lower intake than control birds ($P < 0.05$). Analysis of variance was used to test for significant differences. Data is presented as means ± pooled SE

Results of studies monitoring ATP have reported varying effects of thiamin deficiency on the rat. A decrease in ATP and phosphocreatine in the lateral vestibular nucleus of pyrithiamin-treated rats was reported,[9] but no alteration in ATP was found in the cortex or brainstem in an earlier study.[14] After giving pyrithiamin, high energy phosphate levels were reduced in the brainstem and diencephalon, but were not affected in the cerebral cortex or cerebellum.[26] The results of this paper showed no alterations in midbrain or forebrain (cortical area) ATP concentration, but the brainstem area (hindbrain) had a decrease ($P < 0.01$) in ATP and in the ATP/ADP ratio, which appears to be in agreement with the results of Aikawa and coworkers.[26] This indicates that high energy phosphate concentrations are altered in both dietary and induced pyrithiamin thiamin deficiency. Ischemia, as caused by decapitation into dry ice and acetone, could affect the ATP/ADP ratio. However, all tissue was treated equally. Thus, any differences between groups should not be related to fixation technique. Use of microwave irradiation fixation could decrease the effects of ischemia on this ratio. However, this fixation technique is unavailable.

If there is only a slight excess of alpha ketoglutarate dehydrogenase activity over the brain's needs,[27] then the loss of activity due to thiamin depletion could result in decreased glucose oxidation with a subsequent decrease in ATP synthesis. The medulla-pons area is known to be sensitive to thiamin concentration.[6,28] Thus, the loss of this coenzyme may be related to the alteration in ATP levels found in this area during thiamin deficiency.

Gamma aminobutyric acid is a neurotransmitter with synthesis linked to alpha ketoglutarate in the Kreb's-Citric Acid-TCA cycle.[29] When alpha ketoglutarate concentration increases, the GABA shunt could be used to bypass the decline in succinyl CoA formation caused by decreased alpha ketoglutarate dehydrogenase activity in thiamin deficiency. Brain GABA concentrations are altered in pyrithiamin-treated rats,[30,31] but were not affected in turkeys with a diet-induced deficiency. It may be the regional samples within each of the gross areas tested might provide different results.

Thiamin deficiency affects the Kreb's-Citric Acid-TCA cycle anywhere in the body due to the use of thiamin as a coenzyme. Activities of the three thiamin-utilizing enzymes are decreased in the liver of thiamin deficient rats.[32,33] Due to the problems in pyruvate oxidation and alpha ketoglutarate conversion, the usage of glucose may be slowed. Yet, plasma levels of glucose are not significantly different between the groups. It is possible that systemic hormone action kept glucose within control levels, or the time at which blood glucose levels were monitored was too early to detect a change.

Table 1 Brain analysis of thiamin deficient and control turkeys[a]

Area	ATP	ADP	ATP/ADP	GABA
Forebrain				
Control	1.775 ± .171	1.054 ± .099	1.738 ± .175	1.226 ± .129
T.D.	1.546 ± .171	1.207 ± .099	1.335 ± .175	1.429 ± .129
Midbrain				
Control	2.420 ± .290	4.372 ± .353	.558 ± .058	3.703 ± .213
T.D.	2.326 ± .290	4.915 ± .353	.479 ± .058	3.391 ± .213
Hindbrain				
Control	2.184 ± .180	5.653 ± .527	.412 ± .034	3.022 ± .361
T.D.	1.365 ± .180[b]	5.321 ± .527	.263 ± .034[b]	2.521 ± .361

T.D. = Thiamin deficient; quantity is in μmol/g of brain tissue.
[a] = numbers are means of 10 poults per treatment ± pooled SE
[b] $P < 0.01$

Table 2 Plasma analysis of thiamin deficient and control turkeys*

Test	Mean
Uric Acid	
Control	4.059 ± .366
T.D.	6.813[a] ± .366
Glucose	
Control	312.50 ± 41.5
T.D.	280.28 ± 41.5
β-Hydroxybutyrate	
Control	7.302 ± 3.80
T.D.	15.44 ± 3.80
FFA	
Control	212.33 ± 11.7
T.D.	113.33[b] ± 11.7

* Numbers are means of 10 poults per treatment ± pooled SE
Quantities are in mg/dl for glucose, uric acid, and β-hydroxybutyrate. FFA is in nmol/ml.
[a] = $P < 0.001$.
[b] = $P < 0.0005$.

In the thiamin deficient turkey, the onset of anorexia is rapid. By the fourth and fifth days on experiment, deficient birds have significantly lower feed consumption than the control group, indicating that these birds rely more on bodily stores. In starvation, ketones are produced by the liver. β-hydroxybutyrate and acetoacetate levels are increased in obese humans suffering from starvation.[34] In anorectic turkeys, plasma β-hydroxybutyrate concentration is unaffected, but plasma FFA levels are decreased. This was probably due to exhaustion of fat stores in these young birds.

Conclusion

Uric acid is formed as a result of protein catabolism in birds. Uric acid concentration was increased in the plasma of thiamin deficient birds. This alteration could indicate a reliance on amino acids to provide energy and intermediates for the Kreb's-Citric Acid-TCA cycle.

The Kreb's-Citric Acid-TCA cycle is a key enzymatic system in the body. Since thiamin affects this cycle,[12,13] the cycle itself may be impaired. Acetyl CoA was decreased in the brain of thiamin deficient rats,[35] indicating a decreased conversion of pyruvate to acetyl CoA as catalyzed by the pyruvate dehydrogenase complex. This decline in pyruvate oxidation may cause a decrease in Kreb's-Citric Acid-TCA cycle activity due to less acetyl CoA entering the cycle. The decline in the activity of alpha ketoglutarate dehydrogenase also may decrease Kreb's-Citric Acid-TCA cycle action. As the ability to utilize glucose is decreased, the reliance on nonglucose substrates increases. As the Kreb's-Citric Acid-TCA cycle activity is slowed, less reduced entities may enter the electron transport chain for the formation of ATP. Adenosine triphosphate and the ATP/ADP ratio were decreased in the thiamin sensitive hindbrain area, supporting the concept of altered synthesis. Research has suggested that the Kreb's-Citric Acid-TCA cycle may be regulated, in part, by the ATP/ADP synthesis. The decrease in this ratio may be a regulatory mechanism to increase Kreb's-Citric Acid-TCA cycle activity and, thus, the number of reduced substrate levels entering the electron transport chain. Due to the decrease in thiamin concentration, the system is not able to respond normally. This could allow the bird to become energetically deficient and could allow other symptoms to follow.

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