J. Nutr. Sci. Vitaminol., **29**, 217–225, 1983

Effects of Early Thiamin Deficiency and Subsequent Rehabilitation on the Cholinergic System in Developing Rat Brain

A. B. KULKARNI[1] and B. B. GAITONDE[2]

Department of Biochemistry, Haffkine Institute, Bombay 400012, India

(Received August 13, 1982)

Summary 1. The effects of thiamin deficiency during pregnancy and/or lactation on brain cholinergic system in rat pups were studied. Dietary rehabilitation for a period of 5 weeks from the 28th day was instituted to study possible 'catch-up' in the brain acetylcholine levels.
2. Brain acetylcholine level was found to be significantly decreased on the 21st and 28th days in pups of the dams fed thiamin deficient diet during gestation and lactation, whereas it was decreased on the 28th day in pups of the dams fed thiamin deficient diet during lactation. Activities of cholinergic enzymes remained unaltered in both the deficient groups.
3. Subsequent dietary rehabilitation was found to reverse the deficits in brain acetylcholine levels.
Key Words thiamin deficiency, acetylcholine, acetylcholine esterase, choline acetylase

Beriberi or thiamin deficiency was an important public health problem in the Far East countries till the late sixties according to a Joint WHO/FAO Expert Group (*1*). Endemic beriberi was also not uncommon in the rice eating population of Andhra Pradesh and Tamil Nadu in India (*2*). However, recent studies suggest that the situation has improved and thiamin deficiency is no more a serious public health problem (*3*). Nevertheless, a subclinical or moderate thiamin deficiency has been reported in pregnant women in Malaysia (*4*) and Germany (*5*); in school children in U.S.A. (*6*) and in adults in U.S.A. (*7*), Australia (*8*) and Canada (*9*).

Thiamin deficiency has been associated with specific lesions of the central nervous system (*10*, *11*). It is also associated with significant biochemical alterations in the rat brain (*12*). Encephalopathy due to thiamin deficiency is believed to involve an impairment of cholinergic neurotransmitter function. This has been attributed to the observed decrease in thiamin phosphate availability which might interfere with

[1] Present address: The Institute of Cancer Research and Department of Biochemistry, College of Physicians and Surgeons, Columbia University, Rm. 926, 701 West 168th Street, New York, N. Y. 10032, U.S.A. To whom correspondence should be addressed.
[2] Present address: World Health Organization, New Delhi 110002.

acetylcholine (ACh) synthesis by virtue of decreased production of acetyl-CoA or by altered ATP synthesis (*13*) resulting from decreased activity of thiamin dependent enzyme pyruvate dehydrogenase (*14*).

Several studies have revealed that brain ACh levels are significantly lowered in thiamin deficiency induced by feeding thiamin deficient diet or by administration of thiamin antagonists to adult rats (*15–17*). Thiamin deficiency, however, was not found to have any effect on activities of the cholinergic enzymes, acetylcholine esterase (AChE) and choline acetylase (ChAc), in the adult rat brain (*17*). There is no report of the study on the effects of early thiamin deficiency on the cholinergic system in the developing rat brain. A critical study of the early deficiency deserves immediate attention due to two main reasons. Firstly, rat brain is well known to be vulnerable to nutritional insults during the first 3 weeks of life (*18*). Secondly, beriberi occurs in infants breast-fed by mothers who consume a diet and secrete milk with low thiamin content (*19*). These considerations prompted us to study the effects of maternal thiamin deficiency during gestation and/or lactation on ACh levels and the activities of AChE and ChAc in the developing brain of suckling rat pups. Nutritional rehabilitation of the deficient pups was also attempted and the effects on the brain ACh levels were investigated.

METHODS

Adult female albino rats of proved fertility, weighing 180–200 g (bred at Haffkine Institute), were used for the study. They were divided into four groups, each of which received specific dietary regimen from the 7th day of gestation:

1. G^-L^- group: 20% protein diet without thiamin during gestation and lactation.
2. PFC-1 group: 20% protein diet with normal thiamin levels, pair-fed to G^-L^- group.
3. G^+L^- group: 20% protein diet with normal thiamin during gestation and without thiamin during lactation.
4. PFC-2 group: 20% protein diet with normal thiamin levels, pair-fed to G^+L^- group.

From the 22nd day onward the pups were individually fed on the respective diet. Rehabilitation regimen was initiated from the 29th day by feeding 20% protein diet, with thiamin, *ad libitum*. Water was provided *ad lib.* to all rats. The composition of the diet was same as described earlier (*20*). The deficient diet was analysed for thiamin content by thiochrome method and was found to contain trace amounts of thiamin (*21*).

Pups were sacrificed by decapitation on the 7th, 14th, 21st, 28th or after rehabilitation on 65th day. The whole brain including olfactory lobes was removed, processed further and ACh levels were estimated by frog rectus abdominus bioassay as described earlier (*23, 24*). AChE activity was measured by the method of Ellman *et al.* (*25*). ChAc activity was measured by the method of Fonnum (*26*) using 1-^{14}C-

sodium acetate with a specific activity of 47.69 mCi/mol (obtained from Bhabha Atomic Research Centre, Bombay). Protein levels in the brain were measured by the method of Lowry *et al.* (*27*). The results are expressed as mean ± SEM and statistical significance was assessed by Student's *t*-test (*28*).

RESULTS

Effects of thiamin deficiency during gestation and lactation

Maternal thiamin deficiency during gestation and lactation periods (G^-L^-) was found to result in significant body weight deficits from the 14th day onwards as compared to the pair-fed controls (PFC-1) (Table 1). The deficits in the body weight at 28th day was 25%. Brain weight was found to be unaltered in the deficient pups. Significant changes in the levels of ACh were noticed only at the age of the 21st and 28th day in the deficient group. It is clear from Table 1 that thiamin deficiency during gestation and lactation resulted in the marked decrease to the extent of 24 and 39% in ACh levels at the age of 21 and 28 days respectively. This, however, did not cause any change in the activities of the cholinergic enzymes (Fig. 1).

Effects of thiamin deficiency during lactation

Maternal thiamin deficiency during lactation period (G^+L^-) was found to result into a significant deficit in the body weight from the 14th day onwards to the

Table 1. Effects of maternal thiamin deficiency during gestation and lactation and subsequent dietary rehabilitation on brain acetylcholine levels of the rat progeny.[a]

Age (days)	Dietary regimen	Body weight (g)	Brain weight (g)	Acetylcholine (μg)	
				Per brain	Per g brain
7	PFC-1	8 ± 0.4	0.525 ± 0.015	0.75 ± 0.05	1.43 ± 0.04
	G^-L^-	7 ± 0.4	0.498 ± 0.012	0.71 ± 0.06	1.41 ± 0.08
14	PFC-1	13 ± 0.8	0.832 ± 0.027	1.26 ± 0.12	1.48 ± 0.12
	G^-L^-	10 ± 1.0*	0.746 ± 0.032	1.02 ± 0.14	1.35 ± 0.13
21	PFC-1	20 ± 2.4	1.175 ± 0.040	1.79 ± 0.17	1.51 ± 0.12
	G^-L^-	16 ± 0.9*	1.093 ± 0.032	1.25 ± 0.17*	1.15 ± 0.09*
28	PFC-1	27 ± 2.7	1.275 ± 0.049	2.16 ± 0.23	1.68 ± 0.12
	G^-L^-	21 ± 1.7*	1.152 ± 0.062	1.20 ± 0.16*	1.03 ± 0.08**
56	PFC-1R^+	124 ± 5.6	1.500 ± 0.030	3.37 ± 0.37	2.20 ± 0.21
	$G^-L^-R^+$	114 ± 9.4	1.440 ± 0.035	2.95 ± 0.20	2.02 ± 0.13

[a] Dams were fed 20% protein diet without thiamin (G^-L^-) from 7th day of gestation till weaning, thereafter pups were individually fed. The controls were pair-fed (PFC-1) similarly. Rehabilitation (R^+) was initiated from 29th day. Each group consisted of 8 pups. For ACh estimation two brains were pooled. Values marked with asterisks are significantly different from the controls: $*p<0.05$; $**p<0.01$.

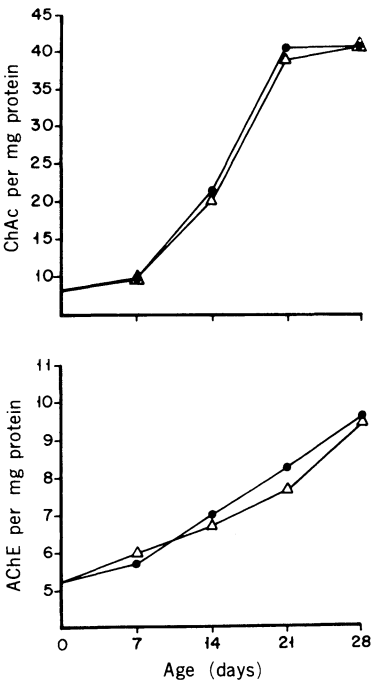

Fig. 1. Effects of maternal thiamin deficiency during gestation and lactation on the activities of AChE (μmol acetylthiocholine iodide hydrolyzed per min) and ChAc (μmol of ^{14}C-acetylcholine formed per hr) in the developing brain of the pups. ●, controls; △, deficient. Each point represents mean ± SE of at least 6 observations.

extent of 35% on the 28th day (Table 2). However, brain weight was not altered as compared to the pair-fed controls (PFC-2). Brain ACh levels were significantly lowered on the 28th day. The deficit in ACh level was 24% as compared to the PFC-2 group. The activities of both the cholinergic enzymes remained unaltered in the G^+L^- group (Fig. 2).

Effects of rehabilitation

Dietary rehabilitation of the pups of the G^-L^- group was attempted to study whether the deficits in ACh levels could be reversed. The deficits in body weight and brain ACh level on the 28th day of age was 20 and 39% respectively in the G^-L^- group. At the end of 5 weeks of dietary rehabilitation, body weight deficit was reduced to 8% and brain ACh levels were found to be similar to that of the controls (Table 1).

DISCUSSION

Reduced gain in the body weight of rats from the G^-L^- group could be due to

Table 2. Effects of maternal thiamin deficiency during lactation on brain acetylcholine of the rat progeny.[a]

Age (days)	Dietary regimen	Body weight (g)	Brain weight (g)	Acetylcholine (μg)	
				Per brain	Per g brain
7	PFC-2	9±0.50	0.553±0.014	0.83±0.03	1.50±0.05 (4)
	G^+L^-	9±0.40	0.545±0.014	0.81±0.04	1.49±0.04 (4)
14	PFC-2	15±0.90	0.877±0.022	1.41±0.14	1.58±0.10 (4)
	G^+L^-	12±1.00*	0.838±0.014	1.28±0.05	1.52±0.08 (4)
21	PFC-2	39±1.30	1.310±0.016	2.23±0.07	1.70±0.04 (8)
	G^+L^-	25±1.50**	1.240±0.016	1.90±0.18	1.53±0.10 (8)
28	PFC-2	59±1.20	1.432±0.016	2.48±0.11	1.73±0.09 (8)
	G^+L^-	38±1.50**	1.364±0.008	2.01±0.05**	1.47±0.03** (8)

[a] Dams were fed thiamin deficient (G^+L^-) diet from the 1st day postpartum and the controls (PFC-2) were pair-fed with G^+L^- group. Number in parenthesis indicates number of observations. Each group consisted of 8 pups per dam and two brain samples were pooled in some cases. Values marked with asterisks are significantly different from the pair-fed controls: $*p<0.05$; $**p<0.01$.

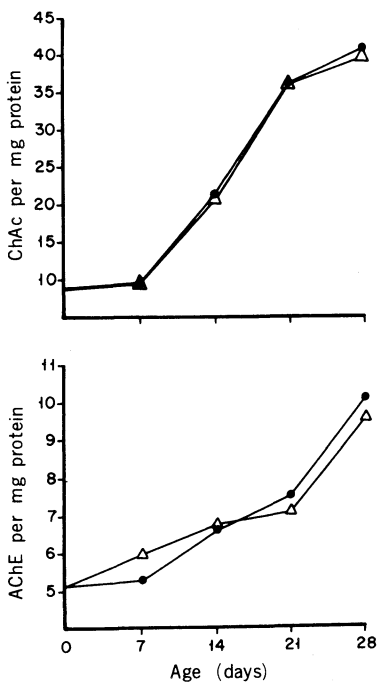

Fig. 2. Effects of maternal thiamin deficiency during lactation on the activities of AChE and ChAc in the developing brain of the pups. Refer to footnote of Fig. 1 for further details.

earlier depletion of maternal thiamin stores and consequently lesser transfer of the same to the fetus. The body weight profiles in the G^-L^- group are comparable to the profiles observed by Trostler *et al.* (*28*). Thiamin deficiency during gestation and lactation is known to depress lactose levels in milk of the deficient dams after 18 days of parturition (*29*). Whereas substantial decrease in percentage of thiamin transfer to pups takes place after the 14th day postpartum (*29*). These two alterations in milk composition of the deficient dams might contribute to a marked decrease in growth of the pups in the G^-L^- group when compared to that of the G^+L^- group. The similar alterations in milk composition of G^+L^- dams may take place much later and thus spare its effect on initial growth period.

Dams of the G^-L^- group consumed less food from the 15th day of pregnancy, *i.e.* following about 13 days of thiamin restriction in the diet. Whereas dams of the G^+L^- group showed a decrease in their food intake at around the 14th day postpartum. This difference in the reduced food intake at different time schedules in the experiment has also contributed to obvious differences in the growth patterns of the pups of the deficient groups. Typical signs of thiamin deficiency as reported earlier (*30*) were apparent by the 22nd day after commencement of feeding thiamin deficient diet. Significant decrease of thiamin levels in liver and also various brain regions were also noticed (*30*). The latter in conjunction with the present observations suggests that thiamin deficiency seems to affect body weight gain but not the brain weights in spite of the reduced levels of the vitamin in different organs including the brain. Similar observations have also been made by other workers (*28*, *29*).

Brain ACh levels were reduced at an earlier age in the G^-L^- group as compared to that in the G^+L^- group. This can be explained on the basis of earlier precipitation of thiamin deficiency in the G^-L^- group as elaborated in the case of difference in the body weight gain of both the deficient groups. Early post-natal thiamin deficiency is known to reduce levels of phospholipids, gangliosides, cerebrosides and cholesterol in the brain of 14 day old rats (*28*, *31*). Presently thiamin deficiency was studied during gestation and lactation and hence it is not possible to conclude whether the brain lipids would be affected in the pups of the dams subjected to thiamin deficiency during lactation period alone.

Brain cholinergic enzyme activity was not altered in the thiamin deficient animals. Heinrich *et al.* also reported unaltered activities of the cholinergic enzymes in the symptomatic thiamin deficient rats (*17*).

There have been recent reports suggesting unaltered ACh levels in brains of thiamin deficient animals (*32–35*). However, thiamin deficiency is known to reduce brain ACh release and utilization (*35*). Moreover, inhibition of pyruvate dehydrogenase activity is also known to decrease incorporation of ^{14}C-label from pyruvate to brain ACh (*36*). Rat brain ChAc is reported to be undersaturated with acetyl CoA in normal animals (*37–39*). Any further depletion of acetyl-CoA availability may prove to be detrimental to normal ACh synthesis. Susceptibility of neurotransmitter synthesis to the availability of precursors is suggested to be mainly

dependent upon the rate limiting biosynthetic enzymes which require better saturation of their substrates (*40*, *41*). The question of such phenomenon occurring in the case of ACh synthesis, particularly in thiamin deficiency, should be most closely looked into.

In conclusion, our observations suggest that maternal thiamin deficiency during gestation and lactation precipitates deficits in brain ACh levels of the rat progeny at an earlier age as compared to maternal deficiency during lactation period alone. The brain ACh deficits in the pups are reversed following dietary rehabilitation for a period of 5 weeks.

We wish to thank Dr. A. J. Baxi for valuable discussions throughout the course of these studies. This research was partly supported by Nutrition Society of India, Grant-in-Aid Award (79-60) to Dr. A. B. Kulkarni.

REFERENCES

1) WHO/FAO Expert Committee (1967): Requirement of vitamin A, thiamine, riboflavin and niacin. FAO Nutr. Meet. Rep. Ser. No. 41, 4 (WHO Tech. Rep. Ser. No. 302, Rome).

2) Patwardhan, V. N. (1960): Nutrition in India, Indian Council of Medical Research, New Delhi, India.

3) Burgess, H. J. L., and Burgess, A. P. (1976): Malnutrition in the Western Pacific. *WHO Chron.*, **30**, 64.

4) Chong, Y. H., and Ho, G. S. (1970): Erythrocyte transketolase activity. *Am. J. Clin. Nutr.*, **23**, 261–266.

5) Heller, S., Salkeld, R. M., and Korner, W. F. (1974): Vitamin B_1 status in pregnancy. *Am. J. Clin. Nutr.*, **27**, 1221–1224.

6) Dibble, M. V., Brin, M., McMullen, E., Peele, A., and Chen, N. (1965): Some preliminary biochemical findings in junior high school children in Syracus and Orondaga County, New York. *Am. J. Clin. Nutr.*, **17**, 218–239.

7) Brin, M., Dibble, M. V., Peele, A., McMullen, E., Bourquin, A., and Chen, N. (1965): Some preliminary findings on the nutritional status of the aged in Orondaga County, New York. *Am. J. Clin. Nutr.*, **17**, 240–258.

8) Wood, B., and Pennington, D. G. (1973): Biochemical assessment of thiamine status in adult Australians. *Int. J. Vit. Nutr. Res.*, **43**, 19.

9) Department of National Health and Welfare (1973): Nutrition Canada, National Survey, Ottawa, p. 113.

10) Peters, R. A. (1967): The biochemical lesions in thiamine deficiency, *in* Thiamine Deficiency, ed. by Wostenholme, G. E. W., Little Brown, Boston, pp. 1–8.

11) Dreyfus, P. M. (1978): Thiamine and the nervous system: An Overview. *J. Nutr. Sci. Vitaminol.*, **22**, 13–16.

12) Gubler, C. J. (1976): Biochemical changes in thiamine deficiencies, *in* Thiamine, ed. by Gubler, C. J., Fujiwara, M., and Dreyfus, P. M., J. Wiley & Sons, New York, pp. 121–139.

13) Mann, P. J. G., and Quastel, J. H. (1940): Vitamin B and acetylcholine formation in isolated brain. *Nature*, **145**, 856–857.

14) Dreyfus, P. M., and Hauser, G. (1965): The effects of thiamine deficiency on the pyruvate decarboxylase system of the central nervous system. *Biochim. Biophys. Acta*, **104**, 78–84.

15) Cheney, D. L., Gubler, C. J., and Jaussi, A. W. (1969): Production of acetylcholine in rat brain following thiamine deprivation and treatment with thiamine antagonists. *J. Neurochem.*, **16**, 1283–1291.

16) Gubler, C. J. (1968): Enzyme studies in thiamine deficiency. *Int. J. Vit. Res.*, **38**, 287–303.

17) Heinrich, C. P. Stadler, H., and Weiser, H. (1973): The effect of thiamine deficiency on the acetylcoenzyme-A and acetylcholine levels in the rat brain. *J. Neurochem.*, **21**, 1273–1281.

18) Dobbing, J. (1968): Vulnerable periods in developing brain, *in* Applied Neurochemistry, ed. by Davison, A. N., and Dobbing, J., Blackwell, Oxford and London, pp. 287–316.

19) Davidson, S., Passmore, R., Brock, J. P. and Truswell, A. S. (1975): Human Nutrition and Dietetics, 6th ed., Churchill Livingstone, Edinburgh.

20) Kulkarni, A. B., and Gaitonde, B. B. (1981): Effects of maternal protein deficiency on brain maturation of the rat progeny. *Bull. Haffkine Inst.*, **9**, 69–74.

21) Kulkarni, A. B. (1979): Brain acetylcholine metabolism in malnourished rats, Ph. D. Thesis, M. S. University of Baroda, India.

22) Kulkarni, A. B., Ved, H. S., and Gaitonde, B. B. (1981): Effects of postweaning prolonged protein deficiency on rat brain enzymes. *Bull. Haffkine Inst.*, **9**, 47–50.

23) Kulkarni, A. B., and Gaitonde, B. B. (1982): Effects of early undernutrition and subsequent rehabilitation on acetylcholine levels in rat brain. *Experientia*, **38**, 377–378.

24) Rajalakshmi, R., Kulkarni, A. B., and Ramakrishnan, C. V. (1974): Effects of undernutrition on acetylcholine levels in rat brain. *J. Neurochem.*, **22**, 119–121.

25) Ellman, G. L., Courtney, K. D., Andres, V., Jr., and Featherstone, R. M. (1961): A new rapid colourimetric determination of acetylcholinesterase activity. *Biochem. Pharmacol.*, **7**, 88–95.

26) Fonnum, F. (1969): Radiochemical micro assay for the determination of choline acetyltransferase and acetylcholinesterase activities. *Biochem J.*, **115**, 465–472.

27) Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J. (1951): Protein measurement with the Folin phenol reagent. *J. Biol. Chem.*, **193**, 265–275.

28) Trostler, N., Guggenheim, K., Havivi, E., and Sklan, D. (1977): Effect of thiamine deficiency in pregnant and lactating rats on the brain of their offspring. *Nutr. Metab.*, **21**, 294–304.

29) Trostler, N., and Sklan, D. (1977): Milk composition and thiamine transfer in thiamine deficient rats. *Am. J. Clin. Nutr.*, **30**, 681–685.

30) Kulkarni, A. B., and Gaitonde, B. B. (1980): Thiamin levels in the brain regions of the thiamin deficient rats. *Bull. Haffkine Inst.*, **8**, 71–74.

31) Dreyfus, P. M. (1976): Thiamine deficiency encephalopathy, *in* Thiamine, ed. by Gubler, C. J., Fujiwara, M., and Dreyfus, P. M., J. Wiley & Sons, New York, pp. 229–239.

32) Hosein, E. A., Chabrol, J. G., and Freedman, G. (1966): The effect of thiamine deficiency in rats and pigeons on the content of materials with acetylcholine-like activity in brain, heart and skeletal muscle. *Rev. Can. Biol.*, **25**, 129–134.

33) Speeg, K. V., Chen, D., McCandless, D. W., and Schenker, S. (1970): Cerebral acetylcholine in thiamine deficiency. *Proc. Soc. Exp. Biol. Med.*, **134**, 1005–1009.

34) Reynolds, S. F., and Blass, J. P. (1975): Normal levels of acetyl coenzyme A and of

acetylcholine in the brain of thiamine deficient rats. *J. Neurochem.*, **24**, 185–186.

35) Vorhees, C. V., Schmidt, D. E., Barrett, R. J., and Schenker, S. (1977): Effects of thiamin deficiency on acetylcholine levels and utilization *in vivo* in rat brain. *J. Nutr.*, **107**, 1902–1908.

36) Gibson, G. E., Jope, R., and Blass, J. P. (1975): Decreased synthesis of acetylcholine accompanying impaired oxidation of pyruvic acid in rat brain minces. *Biochem. J.*, **148**, 17–23.

37) White, H. L., and Wu, J. C. (1973): Kinetics of choline acetyltransferases from human and other mammalian central and peripheral tissues. *J. Neurochem.*, **20**, 297–307.

38) Sollenberg, J. (1970): Determination of acetylcoenzyme A, *in* Drugs and Cholinergic Mechanisms in CNS, ed. by Heilbronn, E., and Winter, A. F., Forsvaretes, Stockholm, pp. 27–32.

39) Shea, P. A., and Aprison, M. H. (1975): The simultaneous measurement of acetyl-CoA, acetylcholine and choline in the same extract from rat brain by a radioenzymatic method. *Trans. Am. Soc. Neurochem.*, **6**, 368.

40) Kaufman, S. (1974): Properties of the pterin-dependent aromatic amino acid hydroxylases, *in* Aromatic Amino Acids in the Brain, CIBA Foundation Symposium, Elsevier, Amsterdam, pp. 85–108.

41) Wurtman, R. J., and Fernstrom, J. D. (1976): Control of brain neurotransmitter synthesis by precursor availability and nutritional state. *Biochem. Pharmacol.*, **25**, 1691–1696.