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Effect of Maternal Phenylalanine Deficiency on the Growth of Their Offsprings

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어미쥐의 Phenylalanine 缺乏이 새끼쥐의 成長發育에 미치는 影響

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= 國文抄錄 =

體重이 200~250g되는 Sprague Dawley 암컷쥐 10마리를 두 그룹으로 나누어서 姙娠末期(third trimester)부터 分娩後 11일까지 低 phenylalanine 食餌(low phenylalanine diet)를 攝取시켜서 새끼쥐의 體重增加 및 血漿과 腦組織의 phenylalanine과 tyrosine 濃度를 測定하였다.

새끼쥐의 體重增加는 對照群에 비해 實驗群은 현저히 減少하였으며, 3일째부터는 두 群사이에 有意的인 差異($p<0.05$)를 나타내었다.

腦무게의 增加는 對照群에 비해 實驗群은 약간 增加되었으며, 實驗 全期間동안 매우 有意的인 差異($p<0.01$)를 나타내었다.

血漿中의 phenylalanine 濃度는 對照群이 實驗群에 비해 약간 높았고, 두 群 모두 점차 減少하다가 增加하는 경향을 보였으며, 5일째에만 有意的인 차 ($p<0.05$)를 보였으나, 전반적으로 血漿中의 phenylalanine 濃度는 食餌의 phenylalanine 양과 相關關係가 없었다.

血漿中의 tyrosine 濃度는 實驗群이 점차로 減少한 반면 對照群은 점차로 增加했고, 5, 9, 11일에는 有意的인 差($p<0.05$)를 나타냈다. 그러므로 血漿中의 tyrosine 濃度는 食餌중의 phenylalanine 양과 positive한 相關關係가 있었다.

腦組織의 phenylalanine은 두 群 모두 增加하는 傾向을 보였으며, 實驗群이 對照群에 비해 약간 낮았으며, 初期에는(1, 3, 5일) 有意的인 差($p<0.05$)를 보였으나 後期에는 有意的인 差가 없었다.

腦組織의 tyrosine은 두 群 모두 增加하다가 減少하는 傾向을 보였으며, 實驗群이 對照群에 비해 약간 낮았고 2, 9, 11일에는 有意的인 差($p<0.05$)가 있었다.

本 硏究를 통하여 姙娠期와 授乳時의 어미쥐의 正常的인 phenylalanine 攝取는 새끼쥐의 正常的인

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成長과 生存에 必須的임을 알 수 있으며, 어미쥐의 endogenous phenylalanine에 의해서 補償되지 않았다.

ABSTRACT

Low phenylalanine diet(0.05%) was given to the Sprague Dawley pregnant rats at the 14 days of gestation and continued until the pups were lactated for 11 days. Body weight, plasma phenylalanine and tyrosine, brain weight, and brain phenylalanine and tyrosine were determined on pups randomly sacrificed at several intervals. Body weight of pups on normal diet (0.36% phenylalanine) gained rapidly while the pups on the phenylalanine deficient diet decreased and did not survive during the period of 11 days. Brain weight of the pups on the phenylalanine deficient diet was significantly lower($p<0.05$) than the normal pups. Phenylalanine deficient diet did not affect the level of plasma phenylalanine of pups, but it seems that there was a positive correlation between the level of phenylalanine in the diet and the plasma tyrosine level. The plasma tyrosine level of pups on the deficient diet was decreased significantly during the period while the pups on the normal diet increased steadily. Phenylalanine and tyrosine level in the brain was lower in pups on the deficient diet than the pups on normal diet but the plasma phenylalanine level was not significantly different in both diets. However, plasma tyrosine level was significantly lower in the pups on the deficient diet than the normal diet at the end of the period.

INTRODUCTION

Normal intake of protein during the pregnancy and lactation is essential to the growth of fetus and the offspring. During the pregnancy, if there is any one of essential amino acid is lacking in the diet, there will be weight reduction and brain damage of the fetus[1)2)].

Phenylketonuria (PKU) is a hereditary form of mental retardation due to a deficiency of the enzyme, phenylalanine hydroxylase, which normally converts phenylalanine to tyrosine in the liver[3)]. A low phenylalanine diet is established therapy for the treatment of PKU which is considered to cause mental retardation by accumulation of phenylalanine in the tissues. Early treatment, within 1-3 months, is said to prevent the brain damage and mental retardation in man[4~6)]. Ford and Berman[7)] reported that there is a positive correlatien between the IQ and phenylalanine hydroxylase activity in phenylketonuric siblings. Additionally, Saugstad[8)] has found intrauterine growth retardation among children born to mothers heterozygous to PKU. Bessman[9)] proposed that phenylalanine hydroxylase supplies tyrosine to the fetal brain and if the mother is PKU heterozygote, there will be limited supply of this tyrosine to the brain and there would be a serious shortage of tyrosine when a fetus is also a PKU heterozygote or when pregnant mother's protein intake is marginal. There is an increased damage of non-specific mental retardation when the pregnant mother cannot eat food because of nausea, vomiting, and toxemia and very limited or no protein intake due to the poor economic situations[9)]. When both mother and the fetus are PKU heterozygote, the risk of being mentally retarded is greater than when either the mother or fetus is heterozygote. A heterozygote mother provide an intrauterine environment unfavorable to the fetus[3)]. Low phenylalanine diet to the preganant rat lowers the activity of phenylalanine hydroxylase but there was a growth retardation and increased production of body lipid[10)11)].

The purpose of this research was to investigate the effect of low phenylalanine diet during the third trimester of pregnancy and the lactation on growth and development of offsprings.

MATERIALS and METHODS

1. Experimental animals

Sprague Dawley female rats (supplied by Seoul National Univ. Experimental Animal Breeding Lab.) weighing 200~250g were mated and put each rat in a separate cage. They were fed commercial rat diet (Jeil Fodder Co.) until the 14th day of gestation. Rats were divided into 2 groups of 5 rats each, control and experimental groups, and fed control and experimental diet ad libitum. Pups were weighed at 1, 3, 5, 7, 9, 11 days of postnatal period and 5 pups randomly selected were sacrificed, and brain weight, plasma phenylalanine and tyrosine, and brain phenylalanine and tyrosine were determined.

2. Experimental diet

The composition of the experimental diet is in Table 1. Experimental diet consists of 10 parts (weight of low phenylalanine casein hydrolysate with no more than 0.1% L-phenylalanine (Lofenalac. Mead Johnson Labs), 6 parts of non-nutritive cellulose and 1 part of sucrose. The diets were mixed well, made into a dough, cut into small pieces and dried in the 50°C over for 24 hours. For the control diet, total of 0.36% L-phenylalanine[12] was added to the experimental diet. The diet was continued until 11 days of postnatal period.

Table 1. Composition of the experimental diet(g/100g)

Total calories	288.3	Thiamin hydrochloride[3]	0.19
Protein	8.8	Riboflavin[3]	0.76
Fat	10.6	Niacinamide[3]	1.69
Carbohydrate	39.43	Vitamin E[4]	2.1
Minerals(ash)[1]	2.94	Vitamin B_{12}[5]	1.9
Calcium	0.38	Folic acid[3]	0.02
Phosphorus	0.29	Biotin[3]	0.01
Iron	0.06	Choline chloride[3]	63.5
Vitamin A[2]	635.3	Pyridoxine hydrochloride[3]	0.21
Vitamin D[2]	169.4	Calcium pantothenate[3]	1.35
Ascorbic acid[3]	17.65		

1. Calcium gluconate, monobasic potassium phosphate, dibasic potassium phosphate, calcium hydroxide, potassium chloride, magnesium oxide and ferrous sulfate
2. USP 3. mg/100g 4. IU 5. μg

Table 2. Body weight in experimental and control groups

Age(days)	Control group(g)	Experimental group(g)
1	4.98±0.73[1](56)[2]	4.14±0.51 (54)
3	5.95±1.01 (51)	4.08±0.51 (45)* (4)[3]
5	7.31±1.41 (46)	4.11±0.45 (34)**(6)
7	9.50±1.88 (41)	3.82±0.33 (20)* (10)
9	11.73±2.60 (36)	3.74±0.47 (9)**(8)
11	13.90±3.23 (31)	3.73±0.59 (4)**(2)

1. Mean±SEM.
2. Number of animals used
3. Number of animals died

* $p<0.05$; compared with the control group by the Student's t-test

** $p<0.01$; compared with the control group by the Student's t-test

3. Blood phenylalanine and tyrosine

Blood phenylalanine was determined by the method of McCaman and Robins[13] and blood tyrosine[14] was also determined.

4. Brain phenylalanine and tyrosine

Whole brain was used after weighing and it was homogenized with 4 volumes of 0.1M phosphate buffer (pH 7.4). After centrifugation of 10,000×g for 20 minutes at 4°C, supernatant was deproteinated with the same volume of 0.6N trichloroacetic acid. Phenylalanine and tyrosine were determined the same way as in plasma.

RESULTS and DISCUSSION

1. Body weight

Normal and low phenylalanine diets were fed to the pregnant rats at 14 days of gestation. Table 2 and Fig. 1 show the body weight of the control and the experimental groups. There was no difference of body weight between two groups when pups were born but the significant difference showed at the third day. Body weight of the pups in experimental group actually decreased during 11 days of postnatal period and most of pups did not survive beyond 11 days. This decrease in body weight explains the excessive

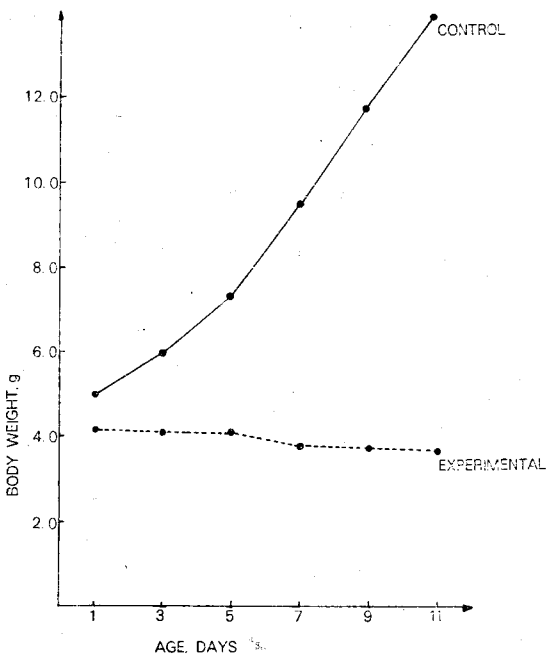

Fig. 1. Body weight in control and experimental groups.

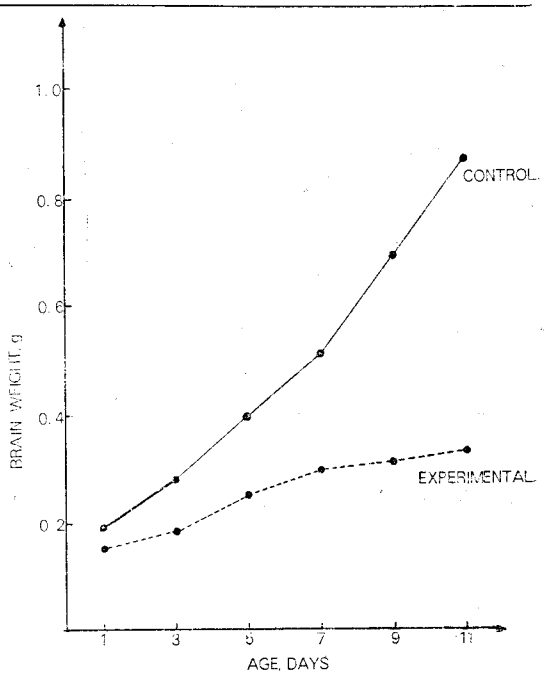

Fig. 2. Brain weight in control and experimental groups.

Table 3. Brain weight in experimental and control groups

Age(days)	Control group (g)	Experimental group(g)
1	0.1938±0.0101[1](5)[2]	0.1572±0.0136(5)**
3	0.2824±0.0433(5)	0.1856±0.0104(5)**
5	0.3977±0.0522(5)	0.2572±0.0167(4)**
7	0.5138±0.0594(5)	0.2956±0.0202(3)**
9	0.6999±0.0774(5)	0.3125±0.0142(3)**
11	0.8699±0.0634(5)	0.3359±0.0185(2)**

1. Mean±SEM.
2. Number of animals used

** $p<0.01$; Compared with the control group by the Student's t-test

degradation of endogenous protein and there might be biochemical changes in the cell when phenylalanine is deficient.[11] When the low phenylalanine diet was supplied to the pregnant rats, there was a growth retardation and death within 2 weeks or cannibalized by the mothers[10].

2. Brain weight

Table 3 and Fig. 2 show the brain weight of the control and the experimental groups. The experimental group had significantly smaller brain than the control group at birth and the difference was getting severe during the 11 days of postnatal period. When any one of the essential amino acids lacks or poor nutrition during the pregnancy, the decreases in brain weight, brain protein, brain DNA, brain myelination and learning ability result the impact on the enzymes in the brain of the fetus (1, 2, 10, 15, 16, 17).

3. Plasma phenylalanine and tyrosine

Table 4 and Fig. 3 show phenylalanine content in the plasma of the control and the experimental groups. Control group showed a little higher plasma phenylalanine than the experimental group but the difference was not significant. Low phenylalanine diet does not seem to have any effect on the plasma phenylalanine level[10][11]. Table 5 and Fig. 4 show tyrosine level of the control and the experimental

Table 4. Blood phenylalanine level in experimental and control groups.

Age(days)	Control group (mg/100ml)	Experimental group (mg/100ml)
1	1.50±0.02[1](5)[2]	1.52±0.01(5)
3	1.57±0.05(5)	1.67±0.01(5)
5	1.35±0.05(5)	1.07±0.03(4)*
7	1.29±0.05(5)	1.06±0.04(3)
9	1.16±0.03(5)	1.16±0.02(3)
11	1.43±0.03(5)	1.36±0.06(2)

1. Mean±SEM.
2. Number of animals used

* $p<0.05$; compared with the control group by the Student's t-test

Table 5. Blood tyrosine level in experimental and control groups.

Age(days)	Control group (mg/100ml)	Experimental group (mg/100ml)
1	2.56±0.04[1](5)[2]	2.66±0.02(5)
3	2.59±0.17(5)	2.38±0.04(5)
5	2.84±0.02(5)	2.10±0.02(4)**
7	2.80±0.02(5)	2.44±0.02(3)
9	3.09±0.03(5)	1.75±0.07(3)*
11	3.15±0.05(5)	1.88±0.24(2)*

1. Mean±SEM.
2. Number of animals used

* $p<0.05$; compared with the control group by the Student's t-test

** $p<0.01$; compared with the control group by the Student's t-test

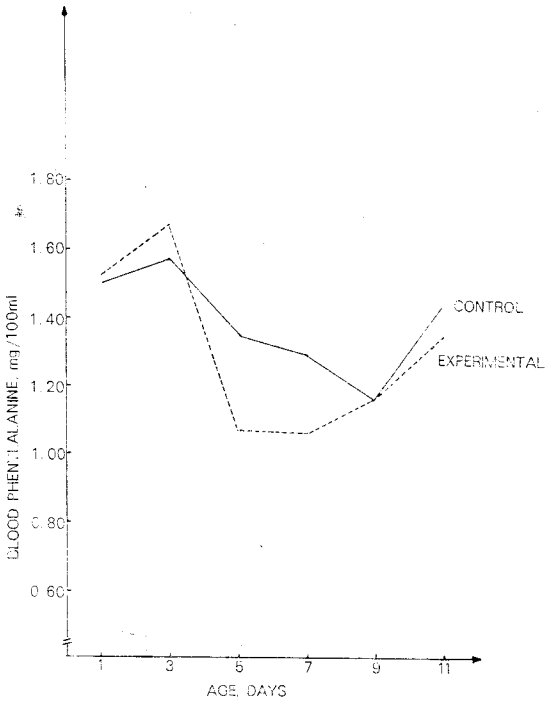

Fig. 3. Blood phenylalanine in control and experimental groups.

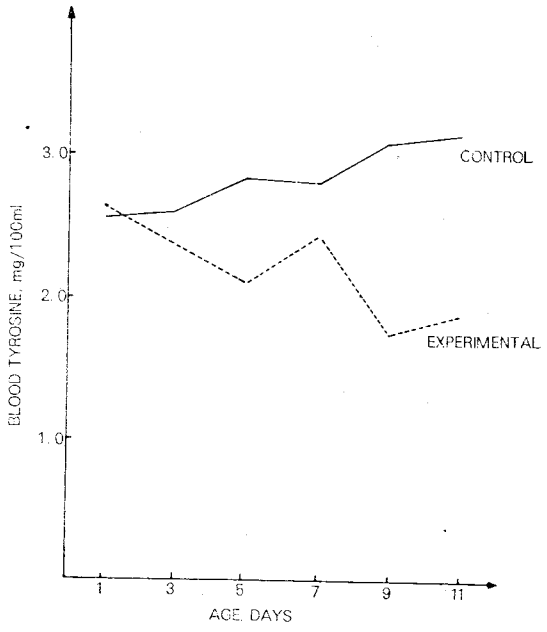

Fig. 4. Blood tyrosine in control and experimental groups.

groups. Pups on low phenylalanine diet showed a steady decrease of plasma tyrosine level, while the pups on normal diet showed a highly significant steady increase during the 11 days of postnatal period.

This suggests a severe effect of the low phenylalanine diet on the level of tyrosine in the plasma. Most of the tyrosine used in the cell processes is converted from phenylalanine and in the low phenylalanine diet, less tyrosine is available from phenyl-

Table 6. Brain phenylalanine level in experimental and control groups.

Age(days)	Control group (nM/g)	Experimental group (nM/g)
1	175.76±3.64[1](5)[2]	132.12±3.03(5)*
3	258.79±3.03(5)	172.73±4.25(5)**
5	247.27±4.85(5)	181.82±3.03(4)**
7	293.94±3.03(5)	393.94±9.70(3)
9	303.03±7.88(5)	284.95±3.03(2)
11	430.30±9.09(5)	351.50±8.49(2)

1. Mean±SEM.
2. Number of animals used
* $p<0.05$; compared with the control group by the Student's t-test
** $p<0.01$; compared with the control group by the Student's t-test

Table 7. Brain tyrosine level in experimental and control

Age(days)	Control group (nM/g)	Experimental group (nM/g)
1	206.60±4.40[1](5)[2]	240.95±3.85(5)
3	269.24±4.95(5)	170.33±2.75(5)**
5	222.53±3.30(5)	239.01±2.75(4)
7	431.87±8.79(5)	456.04±9.89(3)
9	576.92±10.99(5)	335.17±6.60(2)**
11	436.81±9.34(5)	297.81±6.05(2)**

1. Mean±SEM.
2. Number of animals used
** $p<0.01$; compared with the control group by the student's t-test

alanine. It has been estimated that 2/3 of tyrosine synthesized from phenylalanine[18].

4. Brain phenylalanine and tyrosine

Table 6 shows the level of phenylalanine in the brain. Experimental group showed a little lower brain phenylalanine level than the control group but the levels were increasing in both groups during the 11 days of postnatal period. This means that there is no correlation between the low level of phenylalanine in the diet and the level of phenylalanine in the brain. Table 7 shows the level of tyrosine in the brain. There was no significant difference between the control and the experimental groups at the beginning of the experiment but the significant difference was noticed at the end of the 11 experimental days. Phenylalanine level of the brain was 3.2-3.5 times and tyrosine level was 2.3-2.5 times higher than of the plasma levels in both groups. This suggests that there is some correlation between the low level of phenylalanine in the diet and the low level of tyrosine in the brain. Low phenylanine diet resulted in the low tyrosine in the plasma which in return gives less tyrosine available to the brain[19]. Deficient tyrosine in the brain of the newborn pups could have caused the small size of the brain and the excessive breakdown of the endogenous protein due to lack of phenylalanine might have resulted in growth retardation. Normal phenylalanine intake by the pregnant rats during the fetal development and lactation appears to be essential for normal growth and development. This cannot be compensated by the maternal endogenous phenylalanine.

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REFERENCES

1) Zamenhof, S., Hall, S.M., Grauel, L., Van Marthens, E. & Donahue, M.J: *Deprivation of amino acids and prenatal brain development in rats. J. Nutr. 104: 1002—1007 1974.*
2) Zamenhof, S., Van Marthens, D. & Margolis, F.L.: *DNA (cell number) and protein in neonatal brain: alternation by maternal dietary protein restriction. Science 160: 322—323, 1968.*
3) Fujimoto, A., Crawford, R. & Bessman, S.P.: *Relative inability of mother and child to convert phenylalanine to tyrosine: a possible cause of non-specific mental retardation. Biochem. Med. 21: 271—276, 1979.*
4) Friedman, P.A., Fisher, D.B., Kang, E.S. &

Kaufman, S: *Detection of hepatic phenylalanine 4-hydroxylase in classical phenylketonuria. Proc. Nat. Acad. Sci. 70: 552—556, 1973.*

5) Hsia, D.Y. & Holtzmann, N.A: *Medical genetics, eds. McKusick, V.A. & Claiborne, R. (HP Publishing, New York), pp. 237—244, 1973.*

6) Knox, W.E. Phenylketonuria: *The metabolic basis of inherited disease, 3rd., ed eds. Stanb ury, J.B., Wyngaarden, J.B. & Fredrickson, D.S. (New York: McCraw-Hill), pp. 266—295, 1972.*

7) Ford R.C. & Berman, J.L.: *Phenylalanine metabolism and intellectual functioning. Lancet 1: 767—770, 1977.*

8) Saugstad, L.F: *Birthweights in children with phenylketonuria and in their siblings. Lancet 1: 809—813, 1972.*

9) Bessman, S.P. *Genetic failure of fetal amino acid "Justification": common basis for many forms of metabolic, nutritional, and "nonspecific" mental retardation. J. of Pediat. 81: 834—842, 1972.*

10) Dierks-Ventling, C., Wapnir, R.A. & Braude, M.C.: *Biochemical and behavioral effects of commercial 'low phenylalanine' diet in growing rats. Proc. Soc. exp. Biol. 127: 121—127, 1968.*

11) Bessman, S.P., Wapnir, R.A., Pankratz, H.S. & Plantholt, B.A.: *Maternal Phenylalanine deprivation in the rat: Enzymatic and cellular liver changes in the offspring. Biol. Neonat. 14: 107—116, 1969.*

12) Stockland, W.L., Lai, Y.F., Meade, R.J., Sowers, J.E. & Oestemer, G.: *L-phenylalanine and L-tyrosine requirements of the growing rat. J. Nutr. 101: 177—184, 1971.*

13) McCaman, M.W. & Robins, E.: *Fluorimetric method for the determination of phenylalanine in serum. J. Lab. Clin. Med. 59: 885—890, 1962.*

14) Sally, K.: *Biochemical methods in medical genetics, Charles Thomas Publisher, pp. 37—39, 1977.*

15) Dobbing, J., Hopewell, J.W. & Lynch, A.: *Vulnerability of developing brain: Permanent deficit of neurons in cerebral and cerebellar cortex following early mild undernutrition. Exp. Neurol. 32: 439—447, 1971.*

16) Shoemaker, W.M. & Wurtman, R.J.: *Perinatal undernutrition: Accumulation of catecholamines in rat brain. Science 171: 1017—1019, 1971.*

17) Caldwell, D.F. & Churchill, J.A.: *Learning ability in the progeny of rats administered a protein deficient diet during the second half of gestation. Neurology 17: 95—99, 1967.*

18) Bessman, S.P.: *The justification theory-The essential nature of the non-essential amino acids: Nutr. Rev. 37: 209—220, 1979.*

19) Grümer, H.D., Koblet, H. & Woodard, C.: *Phenylalanine metabolism in the phenylpyruvic condition: Distribution, pool size, and turnover rate in human phenylketonuria. J. Clin. Invest. 40: 1758—1768, 1961.*