PJN
ISSN 1680-5194

PAKISTAN JOURNAL OF NUTRITION

ANSInet
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan
Mob: +92 300 3008585, Fax: +92 41 8815544
E-mail: editorpjn@gmail.com

Pakistan Journal of Nutrition 6 (3): 234-237, 2007
ISSN 1680-5194

Effects of Lysine and Essential Fatty Acid Deficiencies on Bone Growth and Development in the Rat

A.A. Odutuga[1] and A.A. Amballi[2]
[1]Department of Biochemistry, Joseph Ayo Babalola University, Ikeji-Arakeji,
P.M.B. 5006, Ilesa, Osun State, Nigeria
[2]Department of Chemical Pathology, Olabisi Onabanjo University, Sagamu, Osun State, Nigeria

Abstract: The effects of Lysine and essential fatty acid deficiencies on bone growth and development were studied in the rat. Forty 19-day old female Wistar rats were divided into four groups of 10 rats each and fed the following diets respectively: (1) a diet adequate in both Lysine and EFA (+Lys+EFA); (2) a diet adequate in Lysine but deficient in essential fatty acid (+Lys-EFA); (3) a diet deficient in lysine but adequate in essential fatty acids (-Lys+EFA) and (4) a diet deficient in both essential fatty acids and Lysine (-Lys-EFA). Each group was placed on its diet for eight weeks. Bones were removed and the lipids estimated. (2) The mean body weights of rats on +Lys+EFA, -Lys+EFA and-Lys- EFA diets were 27.9, 37.2 and 49.7 per cent respectively less than those maintained on the control diet. The lengths and weights of the rat bones were found to decrease in the order: -Lys-EFA> -Lys+EFA> +Lys-EFA> +Lys+EFA. Acidic phospholipids were considerably reduced in proportion in the deficient rat bones. The molar calcium to phosphorus ratio of bones of rats placed on different deficient diets was significantly low when compared with the controls. It is believed that lysine deficiency accentuates EFA deficiency and that both deficiencies impair bone growth and development in the rat.

Key words: Lysine deficiency, EFA deficiency, bone growth

Introduction

Rats maintained on essential fatty acid (EFA) deficient diets exhibit retarded growth, become infertile, develop dermal lesions and have an increased water uptake compared to rats reared on normal diets (Quakenbush *et al*., 1942; Bailey *et al*., 1967; Odutuga, 1977). Similarly, laboratory animals fed on diets deficient in Lysine have been shown to grow poorly and are unable to synthesize protein normally. The body weights, kidney and liver weights of rats fed Lysine deficient diets were considerably reduced (Follis, Jr., 1948; Odutuga and Ogunleye, 1988).

EFA deficiency has been shown to inhibit bone growth and weight. The bones were also hypomineralized (Odutuga, 1982a,b). There seems to be a paucity of information with regards to the effect of Lysine on development of skeletal tissues. This experiment was therefore designed to induce lysine and EFA deficiencies and to compare the effects of the deficiencies on growth and changes in the lipid composition of rat bones.

Materials and Methods

Forty 19-day old rats were fed on EFA deficient diet for one week to deplete them of their EFA stores. The rats were divided into four groups, each containing ten rats and were maintained respectively on the following diets: (a) EFA deficient diet (+Lys-EFA); (b) Lysine deficient diet (-Lys+EFA); (C) diet deficient in both Lysine and EFAs (-Lys-EFA); and (d) control diet, containing adequate Lysine and EFAs (Lys+EFA).

Table 1: Composition of Diet (g/kg)

Components	+Lys+EFA	+Lys-EFA	-Lys+EFA	-Lys-EFA
Groundnut cake[a]	250	250	250	250
Corn starch	516	516	520	520
L-lysine	4	4	-	-
Cellulose	40	40	40	40
Sucrose	100	100	100	100
Corn oil	40	-	40	-
Coconut oil	-	40	-	40
Mineral mixture[b]	40	40	40	40
Vitamin mixture[c]	10	10	10	10

[a]The Groundnut cake was defatted with ethanol and chloroform/methanol (2:1, v/v) (Odutuga, 1982ab); it contained approximately 61.8% crude protein. [b]The mineral mix contained (g/kg diet): $CaCO_3$ (6.54); $CuSO_4$ (0.0072); KI (0.0016); NaCl (4.32); $ZnCO_3$ (0.0176); Ca $(0H)_2$ (19.69); KH_2PO_4 (3.11); $MgSO_4$ (1.13); $KHSO_4$ (7.42); MnO_2 (0.0955) and $Fe_2(SO_4)_4$ (0.38). [c]The vitamin mix contained (g/kg diet): thiamin (0.02); riboflavin (0.03); Pyridoxin (0.10); vitamin B_{12} (0.00003); niacin (0.10); calcium, pantothenate (0.01); p-amino benzoic acid (0.20); myo-inositol (2.00); biotin (0.001); menadione (0.10); folic acid (1.00) and a-tocopherol (0.50); ascorbic acid (0.10); vitamin A acetate (0.04); ergocalciferol (0.4); choline-HCL (2.0) and cellulose (3.31).

The composition of the diets is shown in Table 1. Lipid was removed from the dried groundnut protein as described by (Odutuga, 1982ab) and (Odutuga and Oloyede, 1983). There was no lipid left in the protein. Diets adequate in EFAs contained corn oil, whilst those

Table 2: Effect of diets on body weight (g) of developing rats

Treatment	Initial bodyweight (g)	Final body weights (g)	Group comparison	Differences between final body weight values (%)
+Lys+EFA	32.96 ± 0.67	128.83 ± 4.41	+Lys+EFA vs. +Lys-EFA	27.9
+Lys-EFA	31.10 ± 1.55	92.90 ± 3.90	+Lys+EFA vs. -Lys+EFA	37.2
-Lys+EFA	31.03 ± 1.18	80.95 ± 3.70	+Lys+EFA vs. -Lys-EFA	49.7
-Lys-EFA	32.11 ± 1.94	64.83 ± 3.72	+Lys-EFA vs. -Lys-EFA	30.2
			-Lys+EFA vs. -Lys-EFA	19.9

The results are the mean values for ten rats in each group+SEM

Table 3: Effect of diets on weight (g) of bones of developing rats

Treatment	Weight of Femur	Weight of Tibia	Weight of Humerus
+Lys+EFA	0.35±0.05	0.28±0.06	0.15±0.04
+Lys-EFA	0.22±0.09	0.18±0.05	0.12±0.03
-Lys+EFA	0.20±0.06	0.17±0.06	0.12±0.03
-Lys-EFA	0.18±0.05	0.16±0.07	0.10±0.06

The values are the means of 10 animals in each case+SEM.

Table 4: Effect of diets on length (CM) of bones of developing rats

Treatment	Length of Femur	Length of Tibia	Length of Humerus
+Lys+EFA	2.26±0.05	2.58±0.05	1.93±0.03
+Lys-EFA	2.05±0.04	2.40±0.03	1.70±0.04
-Lys+EFA	2.00±0.04	2.37±0.05	1.69±0.04
-Lys-EFA	1.81±0.07	2.32±0.05	1.50±0.04

The values are the means of 10 animals in each case+SEM.

inadequate in EFA contained hydrogenated coconut oil (Odutuga, 1982a). The diets and water were given *ad-libitum*. The animals were placed on the diet at 19-days of age (average weight 28.9g), blocked by weight and randomly assigned to the different dietary treatments. All rats were pair-fed the quantity of diet equal to the amount consumed by individuals maintained on the diet deficient in both EFAs and lysine (-Lys-EFA) for 8 weeks and were weighed weekly.

The femur, the tibia and the humerus from each rat were removed, cleaned, weighed and the lengths measured.

Lipid analysis: The femur, tibia and humerus were freeze-dried and pulverized in a stainless steel ball-mill to pass through a 240-mesh sieve. The powered sample contained bone and material from the marrow cavity. Pure bone samples were obtained by differential flotation with aqueous cadmium tungstoborate solution (Prout and Shutt, 1970). The samples were washed with water and dried to constant weight. The procedures for obtaining lipid extracts before and after decalcification were as described by (Prout *et al.*, 1973). The lipids extracted before and after decalcification were combined. The total lipid extracts were qualitatively and quantitatively analyzed as described by (Odutuga and Prout, 1974ab).

Analysis of calcium and phosphorus: Known weights of pure bone samples obtained as described above were dissolved in 5N HCl (Odutuga *et al.*, 1975) and aliquots analyzed for calcium (Kingsley and Robnet, 1957) and phosphorus (Bartlett and Robnet, 1957). Ammonium purpurate (Murexide) solution (60 mg/100ml in 70% ethanol) was used to determine the degree of mineralization of the bone samples (Kaufman and Adams, 1954). Analyses of variance were carried out to determine the statistical significance of results.

Results and Discussion

Results of this study have shown that when rats were fed diet deficient in either EFAs, lysine or both, the body weight gain was significantly ($P<0.001$) reduced by 27.9, 37.2, and 49.7% respectively of the control value (Table 2). The body weight gains were 1.71, 1.10, 0.89, and 0.58g/day for the control, EFA deficient, lysine deficient and the double deficient animals respectively. This indicates that Lysine deficiency had a greater effect on the body weights of the animals when compared with EFA deficiency. Lysine is an essential amino acid. It is one of the two completely ketogenic amino acids. Its deficiency will be expected to adversely affect animal metabolism.

The present result show that when rats were fed a diet deficient in lysine and containing no EFAs, the body weight of the rats were reduced by 49.7%. This would probably indicate that EFA deficiency syndrome, might have been aggravated by the lysine deficiency syndrome leading to the impairment of the growth and development of the animals. The significantly lower (10.7%; $P<0.001$) mean body weight of the animals fed the lysine-deficient diet compared with the EFA-deficient animals can be attributed to the unavailability of the amino acid lysine for protein synthesis.

Some similarities exist between changes in body weight and changes in the weights and lengths of the hard tissues (Table 3 and 4) that were studied when the different deficient groups were compared with the controls. For example, the animals decreased in body weight gain by a factor of 0.28, 0.37 and 0.50 respectively for EFA-deficient, Lysine-deficient and the double-deficient animals. The corresponding decrease in the weight gains of femur, tibia and humerus was 0.37, 0.42, 0.49; 0.36, 0.39, 0.43; and 0.20, 0.20, 0.33 respectively. Similarly, the femur, tibia and humerus decreased in length gains by a factor of 0.09, 0.12, 0.20; 0.07, 0.08, 0.10; and 0.12, 0.12, 0.22 respectively. These values suggest that, in this study, the rate of increase in

Table 5: Quantitative LIPID analysis of the bone (femur) of the rats fed lysine and efa deficient diets

Lipid	+Lys+EFA	+Lys-EFA	-Lys+EFA	-Lys-EFA
Cholesterol	19.0±1.41	28.3±0.68	18.9±0.99	30.0±1.62
Cholesterol ester	16.3±0.20	11.1±0.32	15.9±0.38	10.1±0.40
Monoacylglycerol	1.5±0.01	1.4±0.01	1.5±0.04	1.0±0.03
Diacylglycerol	2.7±0.01	1.3±0.01	2.2±0.06	1.1±0.02
Triacylglycerol	12.4±0.21	13.5±0.31	14.2±0.31	15.3±0.63
Free fatty acids	5.1±0.03	4.3±0.03	6.6±0.07	2.9±0.09
Phosphatidyl choline	13.8±0.72	17.5±0.97	13.5±0.88	23.9±1.20
Phosphatidyl ethanolamine	7.4±0.02	5.9±0.03	5.9±0.45	3.4±0.07
Sphingomyelin	2.9±0.01	4.0±0.01	4.1±0.03	2.1±0.02
Phosphatidyl serine	4.5±0.01	2.8±0.01	4.3±0.07	1.9±0.01
Phosphatidyl inositol	5.7±0.02	3.9±0.03	5.9±0.05	3.3±0.01
Phosphatidic acid	3.9±0.01	3.4±0.05	3.0±0.04	2.9±0.01
Diphosphtidyl glycerol	4.3±0.01	2.6±0.04	4.0±0.03	2.1±0.01
Total lipid (% of dry weight of bone)	0.33±0.01	0.33±0.02	0.35±0.01	0.36±0.01

Results are expressed as % of total lipid and are the mean values for 10 bones (analyzed in duplicate)+SEM.

Table 6: The effect of lysine and EFA deficiencies on bone minerals

Treatment	Calcium%	Phosphorus%	Ca/P ratio
Femur			
+Lys+EFA	34.20±0.62	16.50±0.20	1.61±0.02
+Lys-EFA	33.55±0.45	16.35±0.18	1.59±0.03
-Lys+EFA	33.40±0.35	16.40±0.21	1.58±0.02
-Lys-EFA	31.08±0.40	15.90±0.17	1.52±0.02
Tibia			
+Lys+EFA	33.90±0.40	16.30±0.15	1.61±0.03
+Lys-EFA	32.60±0.22	16.40±0.13	1.54±0.02
-Lys+EFA	32.81±0.18	16.20±0.14	1.57±0.02
-Lys-EFA	31.18±0.25	16.10±0.14	1.50±0.03
Humerus			
+Lys+EFA	33.50±0.40	16.20±0.14	1.60±0.03
+Lys-EFA	32.00±0.58	16.25±0.15	1.53±0.02
-Lys+EFA	31.97±0.42	16.00±0.12	1.55±0.02
-Lys-EFA	31.18±0.25	16.10±0.10	1.50±0.03

Results are expressed as%dry weight of tissue, and are the mean values for 10 analyses in each+SEM.

body weight may be a good indication of bone growth. (Odutuga, 1982b) had shown that when rats were fed diets deficient in either EFA or zinc, the bone weights and lengths were considerably reduced. Definitely, the type of diet is a major factor in animal bone growth and development.

Physical signs of EFA deficiency such as dermal lesions around the eyes, feet and the tail that normally appear after about 11-13 weeks (Bailey *et al.*, 1967) on the diet, had started to appear after only 7 weeks on a diet deficient in both EFAs and Lysine. This early appearance of physical signs of EFA deficiency is considered to be due to the accentuation of EFA deficiency by Lysine deficiency.

Differences were observed in the amounts and relative proportions of the lipids extracted from the femur of rats fed the different diets (Table 5). Cholesterol and phosphatidycholine were considerably reduced in proportion in EFA-deficiency and the double-deficiency. The acidic phospholipids, phosphatidylserine, phosphatidylinositol, di-phosphatidylgycerol and phosphatidic acid were significantly (P<0.001) reduced in EFA deficiency. Of the acidic phospholipids, only phosphatidic acid was significantly reduced in Lysine deficiencies (Table 5). The acidic phospholipids have been implicated in the calcification process (Prout *et al.*, 1973; Odutuga and Prout, 1974ab; Odutuga and prout, 1975; Takazoe *et al.*, 1970; Irving, 1973) and can only be extracted after decalcification (Prout *et al.*, 1973). These differences in the lipid composition of bone lipids (caused by EFA or Lysine deficiency or both), observed in the present study, are considered to be responsible for altering the functions of osteo blasts resulting in consequent alteration of bone organic matrix and impairment of bone development and mineralization.

The results of calcium and phosphorus analyses (Table 6) show that the animals fed the control diet, the EFA-deficient diet, the lysine-deficient diet and the diet deficient in both EFAs and Lysine had molar ratios of calcium to phosphorus of 1.60-1.61, 1.53-1.60, 1.54-1.58 and 1.50-1.52 respectively. A molar ratio of calcium to phosphorus of 1.50-1.63 is considered to occur in bone mineral (apatite), depending on how it is formed (Irving, 1973). Although, the present results show that in all the deficiencies, bone mineral was still apatite in nature, yet the molar calcium to phosphorus ratio in these deficient animals was significantly low (P<0.01) when compared with those fed the control diet. This would indicate that less calcium had been deposited when animals were fed these deficient diets.

The murexide staining, however, showed that the bones of animals maintained on a diet deficient in both EFAs and Lysine were totally hypomineralized while those maintained on a diet deficient in either EFAs or Lysine were partially hypomineralized. This indicates impaired or immature mineralization. It might have been induced either by a retardation in the development of the rats or caused by malformed organic matrix.

The deficiency of either Lysine or EFAs resulted in tissue decalcification. The double deficiency increased the degree of decalcification. Lysine is an essential amino

acid and it is entirely ketogenic. It is a precursor of carnitine which transports the essential fatty acid needed in the synthesis of prostaglandins which function in the regulation of intermediary metabolism. Hypomineralization of bones in lysine deficiency reported in this study is believed to be the first report of this kind. The effect of the double deficiency (of both the EFA and Lysine) may be devastating as revealed by the present result of this investigation with pathological signs of EFA deficiency appearing early in the course of the experiment. The present study also suggesting carnitine deficiency due to Lysine inadequacy may indicate that the production of carnitine by methionine alone is not sufficient for normal mammalian lipid metabolism. Adequate amount of preformed carnitine or carnitine from both methionine and Lysine has to be ensured for normal bone growth.

References

Bailey, E., C.B. Taylor and W. Bartley, 1967. Turnover of mitochodrial components of normal and essential fatty acid deficient rats. Biochem. J., 104: 1026-1032.

Bartlett, G.R. and O. Robnet, 1957. Phosphorus assay in cloumn chromatography. J. Biol. Chem., 234: 466-468.

Follis, R.M. Jr., 1948. The Pathology of nutritional disease III. The essential amino acids. Charles C. Thomas, Springfield III, pp: 73-85.

Irving, J.T., 1973. Calcium and Phosphorus Metabol., pp: 71-92 Academic Press, New York.

Kaufman, H.E. and E. Adams, 1954. Water soluble Chelates in histochemical Staining. Sci., 120: 723-732.

Kingsley, G.R. and O. Ronet, 1957. New dye method for direct photometric determination of calcium. Am. J. Clin. Path., 27: 223-230.

Odutuga, A.A., 1977. Recovery of brain from deficiency of essential fatty acids in rats. Biochim. Biophys. Acta, 489: 1-9.

Odutuga, A.A., 1982a. Effects of low-zinc status and essential fatty acid deficiency on growth and lipid composition of rat brain. Clin. Exp. Pharmac. Physiol., 9: 213-221.

Odutuga, A.A., 1982b. Effects of low-zinc status and essential fatty acid deficiency on bone development and mineralization. Comp. Biochem. Physiol., 71A: 383-388.

Odutuga and Ogunleye, 1988. Effect of lysine and essential fatty acid deficiencies on development and lipid composition of rats' liver and Kidney. Nig. J. Pure and Appl. Sc., 2: 110-118.

Odutuga, A.A. and O.B. Oloyede, 1983. Effect of low-magnesium status and essential fatty acid deficiency on growth and lipid composition of chick brain IRCS Med. Sci., 11: 406-407.

Odutuga, A.A. and R.E.S. Prout, 1974a. Lipid analysis of human enamel and dentine. Archs oral Biol., 19: 729-731.

Odutuga, A.A. and R.E.S. Prout, 1974b. Essential fatty acid deficiency on the fatty acid composition of individual lipids from enamel and dentine of the rat. Archs oral Biol., 19: 911-920.

Odutuga, A.A., R.E.S. Prout and R.J. Hoare, 1975. Hydroxyapatite precipitation in vitro by lipids extracted from mammalian hard and soft tissues. Archs oral Biol., 20: 311-316.

Prout, R.E.S. and E.R. Shutt, 1970. Separation of enamel and dentine using cadmium tungstoborate solution. Archs oral Biol., 15: 559-561.

Prout, R.E.S., A.A. Odutuga and F.C. Tring, 1973. Lipid analysis of rat enamel and dentine. Archs oral Biol., 18: 373-380.

Quakenbush, F.W., F.A. Kummerow and H. Steenbock, 1942. The Effectiveness of linoleic, arachidonic and linolenic acids in reproduction and lactation. J. Nutr., 24: 213-224.