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Effect of magnesium and essential fatty acids deficiency on alkaline phosphatase and lactate dehydrogenase activities in the liver and kidney of rats

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ABSTRACT: The effect of magnesium ions (Mg^{2+}) and essential fatty acids (EFA) deficiency on the alkaline phosphatase and lactate dehydrogenase activities in the liver and kidney of rats have been studied. Twenty-one day old female weanling albino rats (*Rattus norvegicus*) with a mean weight of 26.0g were maintained on diets deficient in Mg^{2+}, EFA or both. Control rats were fed diet adequate in magnesium and EFA. The control and the experimental rats were maintained on the various diets for seven weeks. The weights of the tissues of the deficient groups were significantly ($P < 0.05$) less than those of the control. Alkaline phosphatase activity was significantly ($P < 0.05$) increased by one-fifth and one-half in the liver and kidney for the EFA- and the double-deficient rats respectively than those of the control, while it was significantly ($P < 0.05$) reduced to three-quarter and four-fifth of the control rats in the liver and kidney respectively of the magnesium deficient rats. Lactate dehydrogenase activity was significantly ($P < 0.05$) increased in all the tissues of the nutritionally deficient rats. It is considered that both magnesium and EFA are essential nutrients for the normal growth and proper development of rats. EFA, like magnesium, may have a role to play in carbohydrate metabolism due to an increase in LDH activity in EFA deficiency. Both magnesium and EFA deficiency may cause tissues malfunction.

Key Words: Magnesium deficiency; Essential fatty acids (EFAs); EFA deficiency; Alkaline phosphatase; Lactate dehydrogenase.

Introduction

Young rats fed with a very severely magnesium-deficient diet rapidly develop hypomagnesia and overt signs of magnesium deficiency such as skin lesions, hypersensitivity, irritability, hyperemia and retarded growth (Walser, 1967). Magnesium deficiencies are also known to affect the activities of many enzymes involved in phosphate metabolism. An uncoupling of oxidative phosphorylation was observed in cardiac muscle, liver and kidney mitochondria in hypomagnesemic rats (Vitale *et al*., 1957). Alkalnine phosphatase (ALP) declined in serum (Robeson *et al*., 1979) and in duodenum (Nafsey and Schwartz, 1977) of hypomagnesemic rats. The Mg^{2+}-ATPase of the skeletal muscle of hypomagnesemic rats also decreased (Robeson *et al*., 1979).

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Mg^{2+} is required in three of the enzymes involved in gluconeogenesis pathway, namely pyruvate carboxylase (Keech and Utter, 1963), phosphoenolpyruvate carboxykinase (Foster *et al*., 1967) and fructose-1,6-bisphosphate (Mc Glivery, 1964). Magnesium plays a role in the secretion of glucocorticoids, epinephrine, glucagon and insulin (Le Clerg-Meyer *et al*., 1973; Curry *et al*., 1977).

Mg^{2+} in plasma is partly bound to protein and has been shown to be concerned with activation of those enzymes which have thiamine as their prosthetic groups and many enzyme systems, especially the phosphatases (Heaton, 1965) and those that catalyse reactions involving adenosine triphosphate (ATP) (Robeson *et al*., 1979). Thus, magnesium ion is essential for the activity of enzymes like hexokinase, phosphatase, cholineesterase, carboxylase, transphosphorylase, enolase and is thus important in the production of energy in the muscles, carbohydrate metabolism, creatine phosphate synthesis and protein synthesis (Thoren, 1971).

Polyunsaturated fatty acids can be divided into two categories: essential and non-essential. Essential fatty acids (EFA) include linoleic acid (18: 2ω6), and linolenic acid (18: 3ω3) and their respective derivatives. Essential fatty acids belong to a class of nutrients which, in small amounts, are indispensable and in large amounts can modify disease processes.

EFA deficiency has been demonstrated in several mammals including rats, monkeys and man (Neuringer and Connor, 1986). EFA deficiency is characterized by scaly dermatitis, poor growth and increased voluntary food intake and reproductive failure (Sanders, 1988). Changes in balance between ω3 and ω6 fatty acids may affect the physical properties of the membrane-bound enzymes (Morson and Clandinin, 1986). Considering the vital role of magnesium in the synthesis and activity of many intracellular enzymes, and the importance of EFA in maintaining membrane integrity and thus enhancing optimal activity of the membrane-bound enzymes, this study was carried out to investigate the possible effects of Mg^{2+} and EFA deficiencies on the activity of a membrane-bound enzyme (ALP) and a cytosolic enzyme (LDH) in the liver and kidney because of the crucial roles of these organs in the metabolic and active transport of ions in the body.

Materials and Methods

Animals and Diets

Twenty-four female (3 weeks old) wealing albino rats were divided into four groups of 6 rats each. The animals were acclimatized on the diet for a week and then fasted for 24 hours to normalize them for the experiment. They were then placed on the different diets as follows:

(1) The first group consisted of rats fed a diet adequate in magnesium and essential fatty acids. This group is designated +Mg+EFA and henceforth referred to as the control rats.

(2) The second group of rats were placed on a diet rich in EFA, containing 35 ppm/kg of diet. This group is designated as –Mg+EFA and henceforth referred to as the magnesium-deficient rats.

(3) The third group of rats were placed on a diet containing adequate magnesium but no EFA. This group is designated as +Mg-EFA and henceforth referred to as the EFA-deficient rats.

(4) The fourth group of rats were fed a diet containing 35 ppm/kg of magnesium and no EFA. This group is designated as –Mg-EFA and henceforth referred to as the double-deficient rats.

Table 1 shows the composition (g/kg) of the diet in each group. the rats were fed *ad libitum* and deionized water provided over a period of seven weeks. Group feeding was done to ensure animals in each group are subjected to the same condition. Each group of rats was housed in a metal cage at room temperature. At the end of the experimental period, the rats were anaesthetized in a jar containing cotton wool soaked in chloroform. They were then dissected to remove the liver and the kidney. The kidney was

decapsulated. The tissues were then immersed in ice-cold 0.25M sucrose solution. Tissue homogenate was then prepared by cutting a known weight of the tissue with a pair of fine scissors and homogenization was done using pestle and mortar. triton X-100 was added to a final concentration of 1%. All operations were carried out between 0^{o}C and 4^{o}C. The homogenates were stored in the freezer until needed for analysis.

	+Mg+EFA	-Mg+EFA	+Mg-EFA	-Mg-EFA
Soybean meal (defatted)	250	250	250	250
Corn starch	516	516	516	516
Corn oil	40	40	–	–
Coconut oil	–	–	40	40
Sucrose	100	100	100	100
Cellulose	40	40	40	40
DL-Methionine	4	4	4	4
Vitamin mix[1]	10	10	10	10
Mineral mix[2]	40	40	40	40

1Vitamin mix (per kg of diet): Thiamin hydrochloride, 6mg; Pyridoxine hydrochloride, 7mg; Nicotinic acid, 30mg; Calcium pantothenate, 16mg; Folic acid, 2mg; Biotin, 0.2mg; Cyanocobalamin, 0.01mg; Retinol palmitate, 400 IU; Cholecalciferol, 100 IU; α-Tocopherol acetate, 50 IU; Menadione, 0.05mg; Choline chloride, 2g.

2Mineral mix (g/kg diet): CoCl2.6H2O (0.001); CuSO4.5H2O (0.079); MnSO4.2H2O (0.178); KI (0.32), KH2PO4 (15.559); CaSO4 (15.25); NaCl (5.573), ZnCO3 (1.6); FeSO4.7H2O (1.078); MgSO4.7H2O* (2.292).

*Magnesium deficient diet contains 35 ppm/kg.

Enzyme Assay

Alkaline Phosphatase (ALP)

The method described by Bassey *et al*. (1946) which employes the use of p-nitrophenyl phosphate as substrate and modified by Wright *et al*. (1972) was used. Tissue homogenates were incubated with p-nitrophenyl phosphate buffered at pH 10.1 for 10 minutes at 37^{o}C and the reaction was stopped with 1N NaOH. The amount of the hydrolysed product, p-nitrophenol, was then measured spectrophotometrically at 400 nm.

Lactate Dehydrogenase (LDH)

The method of Wroblewski and La Due (1965) was used. The enzyme reduced sodium lactate using NAD as coenzyme. In the course of the reaction, NAD is converted to NADH. The amount of pyruvate liberated from lactate is accurately determined by reacting it with 2,4-dinitrophenylhydrazine in the presence of NADH to produce a red-coloured 2,4-dinitrophenylhydrazone of pyruvic acid whose intensity is proportional to the amount of pyruvate liberated. The reaction was followed spectrophotometrically at 440 nm.

Results and Discussion

The ALP activity of the tissues of rats maintained on the control and the nutritionally deficient diets are shown in Table 2. In all the groups of rats, ALP activity was higher in the kidney than the liver. Bontin *et al*. (1960) reported that the main mammalian organs that have very high ALP activity are those involved in active transport mechanisms, especially the kidney. This may account for the high activity observed in the present study. Compared with the control, ALP activity was significantly increased ($P < 0.05$) in the liver and the kidney of the EFA-deficient and the double-deficient rats. However, the ALP activity of the magnesium-deficient rats significantly reduced ($P < 0.05$) to 78.3% in the liver and 81.4% in the kidney of the control rats tissues.

Table 2: Alkaline phosphatase activity of tissues of the rats fed on diets deficient in magnessium and EFA*.

Dietary Group	Liver	Kidney
+Mg+EFA	32.28 ± 0.81^a	361.0 ± 11.6^a
-Mg+EFA	25.29 ± 2.75^b	293.8 ± 9.94^b
+Mg-EFA	39.43 ± 2.05^c	439.5 ± 10.9^c
-Mg-EFA	48.15 ± 2.01^d	55.24 ± 6.00^d

*Specific activity expressed as nM/min/mg protein. Each value is a mean of 6 determinations ± SEM. Values along the same column with different superscripts are significantly different ($P < 0.05$).

ALP has been reported to be the marker enzyme for plasma membrane (Wright and Plummer, 1974) and is required in certain amount in tissues for proper functioning of the organs (Brain and Kay, 1927). However, when present in large amount as observed in the present study (in EFA and double-deficient rats), it constitutes a threst to the life of the cells which are dependent on a variety of phosphate esters for their vital processes (Butterworth and Moss, 1966), since it may indiscriminately hydrolyze orthophosphate monoesters in the organs. Akanji and Ngaha (1983) reported that ALP is a membrane-bound enzyme and is frequently used to detect diseases or damage to the kidney. This is because ALP is usually present in the brush borders of the renal proximate convoluted tubules (Wright and Plummer, 1974). Thus, the significant increase ($P < 0.05$) in the activity of the kidney enzyme in EFA and double-deficient rats as observed in this study indicates a primary involvement of the kidney cell plasma membrane. In the present study, increase in ALP activity resulting from EFA and double deficiency could be due to increased synthesis of this enzyme in response to tissue damage (Wolf, 1980).

The observation that the liver and kidney ALP activities were significantly depressed ($P < 0.05$) in the magnesium-deficient rats when compared with that of the control may further indicate the essentiality of magnesium as a cofactor for ALP activity (Claude *et al*., 1976). In fact, it has been suggested that magnesium may be essential to the synthesis of ALP (Charles *et al*., 1975). The significant reduction observed in the ALP activity of the tissues of magnesium deficient rats is consistent with the observation of Charles *et al*. (1975) who observed a significant reduction in ALP activities in the bone and plasma of magnesium deficient animals. Furthermore, the same authors observed an increase in the activity in the bone and plasma of magnesium deficient rats when magnesium ion was added to the incubation medium, suggesting that the magnesium ion is not only essential to the synthesis of ALP *in vivo* but also essential to the stabilization of the tertiary structure of the ALP molecule *in vitro* (Charles *et al*., 1975).

Lactate Dehydrogenase

Table 3 shows the lactate dehydrogenase activity of the liver and kidney of rats maintained on the control and the nutritionally deficient diets. The highest activity was recorded in the liver and the least in the kidney. The nutritional deficiency also conferred higher LDH activity compared with that of the control. The highest increase being manifested in the double deficient rats. There was a 3-fold and 5-fold increase in the liver and kidney LDH activity of the EFA-, magnesium- and double-deficient rats. It has been documented that in muscle and liver, lactate dehydrogenase activity exceeds that of all other enzymes of the glycolytic sequence (Abraham *et al*., 1978).

Table 3: Lactate dehydrogenase activity of tissues of the rats fed on diets deficient in magnessium and EFA*.

Dietary Group	Liver	Kidney
+Mg+EFA	0.64 ± 0.04^{a}	$4.6 \times 10^{-2} \pm 0.005^{a}$
-Mg+EFA	1.87 ± 0.22^{b}	$15.2 \times 10^{-2} \pm 0.013^{b}$
+Mg-EFA	1.64 ± 0.31^{c}	$12.6 \times 10^{-2} \pm 0.013^{c}$
-Mg-EFA	2.84 ± 0.28^{d}	$29.2 \times 10^{-2} \pm 0.067^{d}$

*Specific activity expressed as nM/min/mg protein. Each value is a mean of 6 determinations ± SEM. Values along the same column with different superscripts are significantly different ($P < 0.05$).

Rayssiguier *et al*. (1981) have also reported that magnesium deficiency by an unknown mechanism seems to increase glycolysis and to obstruct glycogenesis as shown by increased level of lactate and α-glycerophosphate and by the decreased liver glycogen. It is considered in this study that one of the causes of these phenomena may be the high activity of LDH. This is because lactate is a blind alley in metabolism, once formed there is no means of its further utilization other than reversal of LDH reaction and the formation of pyruvic acid under aerobic conditions. The equilibrium position of the LDH reaction, like that of all reactions involving an alcohol and NAD, strongly favours formation of lactic acid rather than its oxidation, hence during high activity of LDH, glycolysis tends to increase with a net accumulation of lactate.

Furthermore, the high activity of LDH in the EFA- and the double-deficient rats might be able to account for the disruption of carbohydrate metabolism usually noticed in EFA deficient rats.

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