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Changes in Some Serum and Heamatological Indices in Magnesium and Riboflavin Deficiency in Rats

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Abstract: Levels of some serum metabolites were studied in wistar strain rats raised on diets deficient in magnesium (mg) and/or riboflavin (Rb). Though growth was suppressed in the rats fed the vitamin deficient (+Mg- Rb) and magnesium deficient (-Mg +Rb) diets, it was more retarded in those fed the double deficient diets (-Mg -Rb) when compared with the control ($P<0.01$; +Mg+Rb). Significant decreased levels were observed in the total protein and albumin of the Mg and Rb deficient groups when compared with the control ($P<0.01$). Only the double deficient group showed a significant reduction ($P<0.01$) in globulin levels. Creatinine and urea levels in all the groups were reduced when compared with the control while glucose and cholesterol levels were significantly increased ($P<0.01$) except in the (+Mg-Rb) group where a significant decrease ($P<0.01$) in glucose concentration was observed. The hemoglobin (Hb) concentration of the riboflavin deficient group showed a decrease of 19.2% when compared with the control. On the other hand, no marked differences were observed in the erythrocyte levels of the control and deficient groups. These results indicate that magnesium has similar effect as riboflavin deficiency on growth and the selected serum metabolites and that the severity increases with a deficiency of both micronutrients.

Key words: Magnesium, riboflavin, some serum metabolites, some hematological indices

Introduction

Magnesium is an essential intracellular cation involved in more than 300 enzymatic reactions and it acts as a cofactor to adenosine triphosphatases. It is critical in energy requiring processes as protein synthesis and anaerobic phosphorylation (Ahsan, 1997). Serum magnesium concentration is maintained within a narrow range by the kidney and small intestine since under conditions of magnesium deprivation both organs increase their fractional absorption of magnesium. If magnesium depletion continues, the bone store contributes by exchanging part of its content with extracellular fluid.

Magnesium deficiency may result from reduced dietary intake of the ion, malabsorption (Paunier, 1979) and increased renal magnesium wasting (Abbott *et al*., 1994). Alcohol is now known as the most notorious cause of magnesium and riboflavin wasting.

There are experimental and epidemiological data implicating magnesium deficiency in a host of health problems including atherosclerosis (Maier, 2003), ischemic heart disease (Rasmussen *et al*., 1989), arrhythmias (Heigney *et al*., 1997), neuromuscular irritability (Seelig, 1993) and impaired tissue sensitivity to insulin secretion (Barbagallo *et al*., 2003). *In vitro* studies have shown that low magnesium determines endothelial dysfunction, the initiating event leading to the formation of the plaque. Moreover, oral magnesium therapy has been shown to improve endothelial function in patients with coronary artery disease (Maier, 2003). Acute magnesium deficiency results in poor bone formation in immature beagle dogs (Stahlmann *et al*., 2000), cartilage lesions in developing rats (Lozo *et al*., 2003) hypokalaemia and hypocalcaemia (Ahsan, 1997).

Riboflavin, a water soluble vitamin, is available in the form of flavin coenzyme (FAD and FMN) and is found to be important in carbohydrate metabolism. Riboflavin is reported to be associated with many pathological changes. These include hair lesions, diarrhoea (Rivlin, 1996), loss of appetite and anemia (Prentice and Bates, 1980).

Riboflavin deficiency can result from urinary loss of this vitamin as can be observed in respiratory infection (Briglal *et al*., 1999) or from habitual consumption of highly refined and fast foods.

There is abundant information on the effect of either magnesium or riboflavin on different aspects of health. However there is little or no information on the interaction between these two important nutrients. In this study, an attempt is made to investigate the effect of magnesium and riboflavin deficiency on some haematological indices and serum metabolites of developing rats.

Materials and Methods

Thirty-two male wistar strain rats weighing between 26-33g were divided into four groups of eight rats each. The rats were kept in clean cages with mesh floor

Table 1: Composition of basal study diets*

Dietary component	g/kg
Corn starch[1]	516
Casein[1]	250
D-Methionine[1]	4
Cellulose[1]	40
Sucrose[1]	100
Corn oil[2]	40
Vitamin Mix[3]	40
Mineral Mix[a]	10

*By analysis the Mg content of the basal diet was less than 2ppm.
[1]Product from Merck, Darmstadt, FRG.
[2]Product of Taraku oil mill, Taraku, Benue State, Nigeria
[3]Product from Tuco Products, Ont. Canada.
[a](g/kg diet) $CaCo_3$ (6.54); $CuSo_4$ $5H_20$ (0.00072); K. Citrate (9.46); KI (0.0016); NaCl (4.32); Citrate (0.64); $MnSo_4$ (0.055) and $ZnCo_4$ (0.0176).

Table 2: The composition of experimental diets

Diet Group	Basal Diet	Magnesium Supplement mg/Kg diet	Riboflavin Supplement mg/Kg diet
+Mg+Rb	+	0.8	177.6
+Mg-Rb	+	0.8	-
-Mg+Rb	+	0.04	177.6
-Mg-Rb	+	0.04	-

(33x20x13) to prevent coprophagia. All the rats were fed commercial rat pellets (Pfizer Livestock Feeds, Lagos, Nigeria) and deionized water ad libitum for a period of 3 days to acclimatize them with their environment. Thereafter, the rats were fed a riboflavin deficient diet for one week (the basal diet is as shown in Table 1) to deplete them of their riboflavin stores and create uniformity within the population. One group, which served as the control, was maintained on a diet adequate in all nutritive requirements including magnesium (Mg) and Riboflavin (Rb) i.e. (+Mg +Rb). The second group was fed a diet rich in magnesium but deficient in riboflavin i.e. (+Mg- Rb). The third group was fed a diet rich in riboflavin but deficient in magnesium i.e. (-Mg +Rb) while the forth group, also known as the double deficient (DD) group received a diet deficient in both magnesium and riboflavin but adequate in all other nutritive requirements. All rats were maintained on their respective experimental diets (Table 2) and deionized water through out the study period. Body weight gain was measured weekly and after 4 weeks, the animals were fasted overnight and the study terminated. Rats were necropsized under chloroform (BDH, Poole, England) anaesthesia and blood was withdrawn from the aorta into sterilized bottles for serum preparation. The concentration of glucose in the serum was determined by the glucose oxidase method (Trinder, 1969), concentration of creatinine was determined with alkaline picrate (Jaffe reaction) similar to the standard manual procedure for blood and urine (Henry *et al*., 1974), urea nitrogen concentration in serum was measured spectrophotometrically using the method of Marsh *et al*., 1965 while Serum cholesterol was determined by the enzymatic end -point method (Richmond, 1973), in each case, using already prepared kit from Randox Industries Ltd UK and adhering strictly to the manufacturer's instructions.

Total protein was determined by the Biuret method (Henry *et al*., 1974) while serum albumin levels were determined as previously described by Doumas *et al*., 1971, in both cases using already prepared kits from Randox Laboratories Ltd UK. The globulin concentration was then obtained by subtracting the albumin concentration from that of the total protein.

Haemoglobin and erythrocyte concentrations were determined using Compur M. 100 mini photometer from Compur-Electronic GmbH, West Germany.

When added magnesium content was supplied as magnesium carbonate. The low magnesium diet contained 0.04mg magnesium/kg diet in order to avoid severe magnesium deficiency (Zieve *et al*., 1997) while 0.8mg magnesium/kg diet was added to the diet containing adequate magnesium. The riboflavin rich diet was supplemented with 177.6mg riboflavin /Kg diet.

The results were expressed as means ± SEM. Analysis of variance ANOVA was used to test for differences between all the groups while Duncans multiple range tests was used to test for significant differences between the means (Sokal and Rohlf, 1969).

Results

Some pathological and physiological changes were observed in the rats fed the deficient diets. These include hair lesions, hunched posture and loss of appetite.

Fig. 1 shows the growth index of the rats during the four weeks of feeding the experimental diets. The growth curve for the rats on the control diet showed substantive weight gain, rising sharply on the 7th day. The growth curves of the rats fed either of the deficient diets on the other hand showed lesser weight gain although they also exhibited a sharp increase in growth rate on the 7th day. However the growth curve after the first week in the rats maintained on the double deficient diet was not as steep as those of any of the other groups of rats. This shows a more severe growth retardation in the rats fed the double deficient diet

Mean body weight gains as well as comparison between final body weight values of the different groups are shown in Table 3. The body weights of the rats maintained on diets that are deficient in either riboflavin or magnesium were considerably reduced ($P<0.01$) when compared to the control group. The results also indicate that the body weight of the rats fed the double deficient diet (- mg -Rb) decreased more than (- mg) or

Table 3: Body weight (g) of rats in all the study groups

Dietary groups	Initial body wt. (g)*	Final body wt (g)*	Group comparison	%Differences between final body wt value.
+Mg+Rb	29.02±2.6	160.25±1.3a	+Mg+Rb vs +Mg-Rb	17.9
+Mg-Rb	30.50±2.1	131.50±2.3b	+Mg+Rb vs -Mg-Rb	28.6
-Mg+Rb	30.13±1.2	124.75±1.2b	+Mg+Rb vs-Mg+Rb	22.2
-Mg-Rb	30.75±1.5	114.38±1.0b	+Mg-Rb vs -Mg+Rb	5.1
			-Mg+Rb vs -Mg-Rb	8.3
			+Mg-Rb vs -Mg-Rb	13.0

*Values are the means of eight rats ±SD. Values on the same column with different alphabets vary significantly a: P >0.05: b: P < 0.01

Table 4: Results of hematological examinations of rats maintained on the 4 study diets

	Group			
Serum metabolites mg/100ml	+Mg+Rb	+Mg-Rb	-Mg+Rb	-Mg-Rb
Total Protein	106.0±2.7^{a}	57.3 ± 2.5^{c}	49.5 ± 2.0^{c}	45.8 ± 2.0^{c}
Albumin	78.9 ± 2.4^{a}	30.3 ± 0.5^{c}	26.0 ± 2.0^{c}	30.8 ± 1.2^{c}
Globulin	27.9 ± 0.3^{a}	27.0 ± 2.0^{a}	23.5 ± 0.1^{b}	15.0 ± 0.8^{c}
Creatinine	0.7 ± 0.04^{a}	0.5 ± 0.02^{a}	0.2 ± 0.02^{b}	0.5 ± 0.01^{a}
Glucose	71.70 ± 2.0^{a}	58.36 ± 1.3^{c}	88.0 ± 1.5^{b}	80.0 ± 1.6^{a}
Urea	52.9 ± 1.4^{a}	46.6 ± 1.3^{a}	43.2 ± 1.3^{b}	39.4 ± 1.4^{c}
Cholesterol	52.5 ± 1.5^{a}	65.2 ± 0.9^{b}	61.0 ± 1.2^{a}	61.4 ± 1.1^{a}
Hemoglobin (g%)	13.0^{a}	10.5^{b}	13.5^{a}	11.5^{b}
Erythrocyte (million/mm^{3})	4.0^{a}	4.2^{a}	4.0^{a}	4.3^{a}

*The values are the means of eight rats ± SEM. Values with the same alphabets do not differ significantly. a: P > 0.05; b: P < 0.05; c: P < 0.01

(- Rb) deficient groups (P<0.01). However, rats placed on the magnesium deficient diet showed significant reduction (P<0.01) in weight by 5.1% when compared to those maintained on the riboflavin deficient diet.

There was significant reduction (P<0.01) in the serum total protein and albumin levels in all the experimental groups when compared to the control group (Table 4). However, only the globulin concentration of the group maintained on the double deficient diet was observed to be significantly reduced (P<0.01) when compared to the control.

The levels of Creatinine in the sera of the -Mg+Rb group showed a significant reduction (P<0.01) compared with the control. Except for the rats in the +Mg-Rb group, all the other groups showed a marked increase in glucose concentration compared to the control. The study thus reveals that feeding rats with diets deficient in either magnesium alone or magnesium and riboflavin results in hyperglycaemia. There was a significant increase (P<0.05) in the cholesterol levels of the rats in the +Mg-Rb group compared with the control. The hemoglobin concentration of the rats fed the riboflavin deficient and double deficient diets were significantly (P<0.05) reduced compared to that of the control. On the other hand no marked difference was observed in the erythrocyte concentration of all the dietary groups compared with the control.

Discussion

Magnesium and riboflavin contribute in no small measure to the maintenance of a healthy life. Studies have documented the role of a deficiency in either Mg or Rb. However, there is no report on a deficiency of both micronutrients in rats. This study reports changes in some serum metabolites in rats fed a magnesium and riboflavin deficient diet.

The resemblance in growth curves for the four groups of rats before the 7th day of feeding the experimental diet may be attributable to adaptation of the rats to the new diet during this period. Adelekan and Thunham (1986) reported loss of appetite concomitant with impaired body weight gain in riboflavin deficient rats, even at the early stages of feeding the deficient diet. Also Zieve *et al*. (1997) reported growth retardation in rats maintained on magnesium deficient diet. The similar growth curve in the first seven days of feeding the respective diets may not also be unconnected with the use of available magnesium and riboflavin in the diet prior to the commencement of feeding with the experimental diet. The present study however, has shown that though the growth curves for the four groups were very similar, growth was more retarded in the rats fed the magnesium deficient than the riboflavin deficient diet. This result is expected as magnesium is required for virtually every step in protein synthesis (Freude *et al*., 1978) and riboflavin deficiency leads to a disruption of all cellular reactions that are linked with enzymatic processes in which flavo proteins participate (Rhoads *et al*., 1941).

The growth pattern observed correlates with the pattern

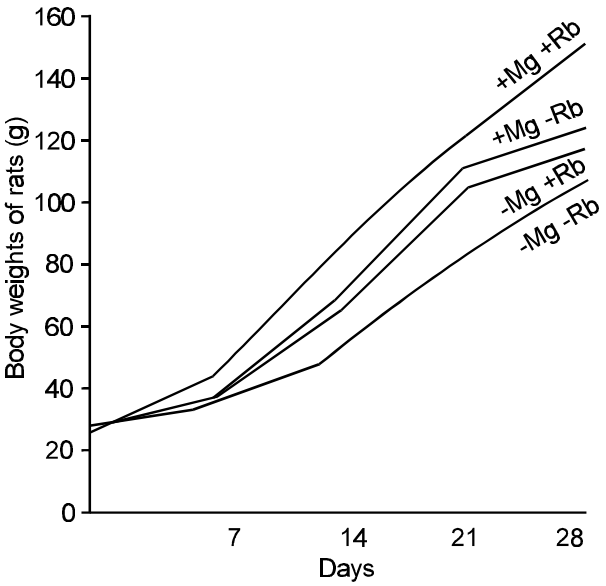

Fig. 1: Rate of growth of different groups of rats fed on control manesium deficient, riboflavin deficient and combined deficiency diets

exhibited in the serum levels of total protein and albumin. This lower level of serum total protein and albumin in all the rats maintained on the deficient diets is probably due to redistribution of protein fraction as a result of increased capillary membrane permeability or increased catabolism (Nassir *et al*., 2001) or decreased protein synthesis. The latter can be attributed to malnutrition resulting in an inadequate supply of dietary nitrogen. Zieve *et al.*, (1997) had earlier reported impaired protein synthesis in magnesium deficient rats. The result obtained in this study may not also be unconnected with the drop in appetite in all the groups maintained on the deficient diets. The possible effect of these micronutrients on protein metabolism may help explain the low urea levels observed in the rats fed with the diets deficient in either magnesium alone or those deficient in both micronutrients (Table 4).

The significant reduction in the serum albumin concentration observed in the rats fed with the micronutrient deficient diets (Table 4) would affect the important function of transportation of nutrients to tissues and would affect growth. It is not surprising therefore that there was an observed decrease in weight of these rats. As the liver almost exclusively produces albumin, these findings would suggest that lack of either of these micronutrients might severely alter metabolic processes in the liver. The basal diet used in this study was not completely devoid of magnesium thus the lack of significant change in serum albumin level observed in the rats fed the magnesium and riboflavin deficient diet suggests that either of the micronutrients may have complimentary effect on albumin production.

Serum creatinine level has a direct correlation with muscle mass and kidney function. The observed low serum creatinine level in the rats fed with the diet deficient in magnesium therefore suggests that this micronutrient affect body mass and/or kidney function. If it affects body mass, it will explain the observed low weight in the rats fed with this diet. As low body mass is mostly correlated with protein, magnesium deficiency may alter body metabolism in ways which decrease protein synthesis and can account for the observed low serum protein and albumin in these rats.

High Cholesterol levels were observed in all the deficient groups studied. This increase is probably due to increased mobilization of cholesterol from the liver (Goswami and Sadhu, 1961) or the involvement of magnesium deficiency in altering the plasma low density lipoprotein (LDL) levels (Maier, 2003; Rasmussen *et al*., 1989). Hypercholesterolaemia is almost always due to raised plasma LDL concentrations (Mayne, 1994).

The hyperglycaemia occasioned by riboflavin and magnesium deficiency may be related to the involvement of these micronutrients in carbohydrate metabolism. Riboflavin in the form of flavin coenzyme, FAD and FMN are important in glucose metabolism, so in the absence of riboflavin, glucose metabolism is impaired. Also reports (Barbagallo *et al*., 2003) indicate that poor intracellular magnesium concentration results in the impairment of insulin action. This is because intracellular magnesium plays a key role in modulating insulin mediated glucose uptake. The increase in plasma glucose levels in the two magnesium deficient groups reported in this study could infer an impairment of insulin function.

Riboflavin deficiency may impair iron absorption, increase intestinal loss of iron and/or impair iron utilization for the synthesis of hemoglobin (Powers, 1995). This study shows that hemoglobin concentration decreases in the rats maintained on riboflavin deficient and the double deficient diets. The hemoglobin concentration of the riboflavin supplemented (-Mg+Rb) group was similar to those of the control, showing the ability of riboflavin to bring about a general improvement on the hematological status of magnesium deficient rats (Charoenlarp *et al*., 1980; Powers *et al*., 1983). On the other hand, no statistical differences were observed in erythrocyte concentration of rats in all the study groups.

In summary, this study shows that magnesium deficiency has similar effects as riboflavin on growth and the indicated serum metabolites in rats. These, as well as the observation that the double deficiency aggravates the symptoms of riboflavin deficiency, have led us to suggest that magnesium is essential for the normal metabolism of riboflavin.

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