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EFFECTS OF RIBOFLAVIN DEFICIENCY IN RATS ON SOME ASPECTS OF IRON METABOLISM

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SUMMARY

Female Norwegian Hooded rats were given a diet low in riboflavin prior to pregnancy and during gestation and lactation. Each animal had a pair-fed control receiving a diet adequate in riboflavin. The ability of liver mitochondrial preparations to mobilise iron from ferritin was measured in the dams and their pups. The rate of mobilisation was reduced in the deficient animals relative to their controls and sigificantly so for the pups. The data support the hypothesis that riboflavin is an important factor in controlling iron utilisation.

INTRODUCTION

It has long been recognised by haematologists that dietary iron availability is not the sole factor determining the incidene of microcytic hypochromic "iron-deficiency" anaemia (Harris, et al., 1974). Over recent years some attention has been given to the possible role of the water-soluble vitamin rioflavin. in determining iron movement in the body. Human studies have suggested that riboflavin can increase the effectiveness of an iron supplement in correcting microcytic hypochromic anaemia (Powers, et al., 1990) and that ribovlavin can reduce the decline in haematological status sometimes assocated with pregnancy (Decker, et al., 1987). Studies with laboratory animals have indicated that the importance of riboflavin may lie in its involvement in the mobilisation of iron from a carrier protein ferritin (Sirivech et al., 1992; Zaman et al., 1987). However, such studies have often failed to define the extent of riboflavin deficiency achieved in the animals used and the practice of pairfeeding control animals. A prerequisite o ascribing an effect specifically to a deficiency of riboflavin has been neglected.

MATERIAL AND METHODS

An experiment was designed to investigate the role of riboflavin on iron metabolism by studying the process of iron release from ferritin by liver mitochondria.

Animals: All animals were housed individually in metal cages. At the begining of the study each rat was fitted with a plastizote collar in order to prevent coprophagy but so as not to inhibit feeding. period of several days acclimatisation to the collars elapsed before putting the rats on their study diets.

Diets: All animals received a basal diet (diet A) containing 20% protein and no riboflavin. Riboflavin-deprived rats received 0.12 mg B2/kg diet (diet B). Each B_2-deprived animal was fed ad lib. and had a pair-fed control animal receiving diet A supplemented with 15 mg B_2/kg diet (diet C).

Protocol: 7 week old female Norwegian Hooded rats, mean weight (8) g, were fed diets B or C for 5 weeks. Collars were removed and animals were mated. After 3 weeks of lactation dams and pups were killed and liver mitochondria were prepared for iron mobilisation studies. Liver samples were frozen for flavin analyses, and red blood cells stored for measurement of erythrocyte glutathione reductase activation coefficient (EGRAC).

Iron mobilisation: Iron mobilisation was measured by a considerable adaptation of a method de-

scribed by Ulvik and Romslo (Ulvik, et al., 1989). The principle behind the measurement is that in the presence of an active flavin mononucleotide (FMN) dependent oxidoreductase, ferric iron in ferritin will be reduced, released, and chelated by bipyridyl. Bipyridyl-Fe has an absorbance maximum at 530 nm. Mitochondria were prepared in 5 mM Hepes buffer, PH 7.4 (Ulvik, 1981) and were suspended at a protein concentration of 1-2 mg/ml in the following medium: 0.25 M sucrose. 10 mM-Hepes, PH 7.4 1 mMsuccinate. 1.25 mM-bipyridyl, 50 uM-FMN, 1 mM-ATP, 1 mM. $MgCl_2$. The medium was flushed with N2 for 2 mins and transferred to the sealed chamber of an O_2 -ele-ctrode equilibrated at 37°C. When anaerobiosis was reached ferritin was added to give an iron concentration of 0.45 mM. aliquots were removed in duplicate at time zero and at 10 and 20 mins after ferritin addition and solubilised in an equal volume of 1% TRITON-X-100. Abosrbance was read at 530 nm against a reagent blank. The amount of iron liberated was determined using an extinction coefficient of 8.65 mM^{-1} cm^{-1}. Protein concentrations were measured using a modified lowry technique (Bensadown, et al., 1986).

Riboflavin Status: Riboflavin status was assessed by measuring the erythrocyte glutathione reductase. activation coefficient. The assay was performed on the Bobas Bio Autoanalyser using a modification of the established spectrophotometric assay. Erythrocytes were preincubated at 37°C for 30 mins at a haemoglobin concentration of approximately 150 mg% with or without added flavin adenine dinucleotid (FAD) at a final concentration of 2 uMolar. The reaction was monitored at 37°C in the presence of 0.59 mM-glutathione and initiated by 132 uM reduced nicotinamide adenine dinucleotide phosphate. Liver flavins were measured fluorimetrically (Baker, et al., 1991).

RESULTS

The extent of the deficiency of riboflavin achieved is illustrated in Table 1 in terms of (EGRAC) values and liver flavin concentrations. The riboflavin deprived dams had a mean (EGRAC) of 1.67, constituting a biochemical riboflavin deficiency. The pups born of these dams were also clearly biochemically riboflavin-deficient. The concentration of liver flavins, expressed both as (FMN + riboflavin) and as FAD alone, were significantly lower in the riboflavin-deprived animals than in their respectively controls.

Iron mobilisation expressed as n moles iron mobilised/min mg protein, showed some association with riboflavin status. Thus, as presented in Table 2, the lactating dams and their pups showed a lower rate of iron mobilisation than their Controls.

Table. 1: Liver flavins and erythrocyte glutathione reductase activation coeffient.

Liver flavins ug/g liver					
EGRAC		FMN+B_2		FAD	
P	D	P	D	P	D
DAMS 1.17+0.053	*** 1.67+0.042	5.42+0.51	*** 2.92+0.23	2.21+0.72	** 8.74+1.52
PUPS 1.30+0.023	*** 1.77+0.030	6.76+0.33	3.59+0.22	3.28+0.75	*** 4.06+0.66

Means and SEM for EGRAC and liver flavins are shown.
P: Pair fed.
D: Riboflavin-deficient.
Significance of difference between pair-fed and control animals.
** P < 0.01 *** P < 0.01

Table. 2: Iron mobilisation from ferritin by liver mitochondria.

Nomol Fe min mg protein	
B_2. deficient	Pair Fe.d
DAMS 0.16±0.029 PUPS 0.10+0.22	0.26±0.053 *0.25+0.046

Values are mean + SEM for nmoles Fe mobilised/min./mg mitochondria protein.
Significance of difference between deficient and pair-fed animals.
* $P < 0.05$.

The reduction reached statistical significance for the pups ($P > 0.05$). The rate of iron mobilisation in the dams and pups showed firm correlations with the three expressions of riboflavin status.

DISCUSSION

The results indicate that a biochemical deficiency of riboflavin can impair the rate of removal of iron from ferritin by a mitochondirial preparation in vitro.

In addition. The correlations demonstrated between the rate of iron mobilisation and riboflavin status suggest that the extent of the impairment will be determined by the degree of riboflavin deprivation. It is by no means established either that an FMN--dependent oxidoreductase is the predominant means of removing ferric iron from ferritin in vivo and indeed precisely what role ferritin plays in intracellular iron movement. It is however, clear that of the small molecular weight reductants that can mobilise ferritin iron in vitor the kinetics of flavin involvement are particularly favourable (Sirivech, et al., 1992). FMN-dependent ferriductase activity has been reported to occur in homogenate, microsomal, and mitochondrial preparations of rat liver (Ulvik, 1979; Zaman et al., 1987). Of particular interest as regards this study is the fact that riboflavin status apparently influences iron mobilisation in a system in which FMN is supplied in excess. Under such conditions at least two factors are likely to influence the rate of iron mobilisation:

1- Respiratory rate of mitochondria.
2. Concentration of ferriductase.

The efficiency of FMN as reductant is dependent in part on its ability to undergo cyclic redox reactions and to transfer reducing equivalents from the respiratory chain to ferritins studies by Ulvik and Romslo (Ulvik, 1979) have indicated that flavins drain reducing equivalents from the mitochondria at the level of succinate dehydrogenase. The ability of the mitochondria to generate reducing power would therefore be expected to influence the iron release process. The experience in this laboratory is that hepatic mitochondria from riboflavin deficient adult rats have normal rates of O_2 uptake with glutamate as substrate (Duerden, personal communication).

The possibility that the concentration of a vital FMN-oxidoreductase limits the rate of iron mobilisation is supported to some extent by the correlations observed between the mobilisation rate in vitro and the concentration of liver flavins. It is recognized that in the absence of their confactor some enzymes are less stable. It is possible that during a period of dietary deprivation of riboflavin, with a consequent lowering of FMN concentrations in the liver, some loss of functional FMN oxidoreductase occurs.

The physiological significance of riboflavin involvement in iron mobilisation from ferritin is to some degree dependent on the precise role of ferritin in iron economy. It is certainly not yet established whether ferritin functions as an obligatory intermediate in the cytosolic transport of iron but several pieces of research have suggested that ferritin may have such a role (Mazur, et al., 1963; Speyer et al., 1979).

The demonstration that iron mobilisation in a specific in vitro system is influenced by riboflavin status does not of course indicate in what way this might manifest itself in haematological terms. Non-pregnant non-lactating female rats, which were riboflavin- deficient but less severely so that those described here, showed a trend towards reduced incorporation of Fe into haem, suggesting that imparied Fe mobilisation from ferritin may have functional consequences. Further investigations are in progress to confirm these preliminary observations.

Clearly further studies need to be performed in order

to define the precise role of flavins in iron metabolism but data presented here support the hypothesis tht riboflavin status infuences iron metabolism by interfering with iron mobilisation steps.

REFERENCES

Baker, H. Frank O. (1991): the effect of Riboflavin on serum iron. In "Riboflavin" Ed. Rivlin R.S., P. 59, Plenum Press, N.Y.

Bensadoum, A., Weinstein, D. (1982): Riboflavin and Iron Anal. biochem. 70, 241.

Decker, K. Dotis, B., Glatzle, D., Hinsselmann, M. (1987): Metabolism and Req. of iron in the human Nutr. Metab. 21. 17.

Harris, J.W.; Kellermeyer, R.W. (1974): In "The Red Cell", P. 110 Harvord University Press.

Mazur, A.; Carleton, A. (1963): The determination of ribo flavin. J. Boil. Chem. 238, 1817.

Powers. H.J., Bates, c.J., Dlerden 91988): Human Nutrition Clinical Nutrition. In Preparation.

Speyer B.E. Fielding J. 91979): In "Mineral Metabolism Brit. J. Haem. 42, 255.

Sirviech. S., Frieden. E., Osaki. S., (1992): Riboflavin Relationships in the Dev. of Anemia Biochem. J. 143, 311

Ulvik, R. Romslo, I., (1978): Absorption & Metabolism of Iron. Biochem. et Biophys. Acta 542, 251.

Ulvika, R., Romslo I., (1979): Amino Acids contents of foods Biochem. et. Biophys. Acta 588, 256.

Ulvik, R.J. (1981): In "Essential Amino Amino Acids FEBS Lett. 132, 281.

Zaman, Z. Verwilghen R.L. (1987): Evaluation amino acids Biochem. Soc. Trans, 5, 306.