Biokemistri Vol. 5, No. 1, June, 1995
Printed in Nigeria

0795-8080/95 $3.00 + 0.00
Klobex Academic Publishers

BKR9505/5104

# COMPARATIVE STUDIES OF IRON AND ZINC DISTRIBUTION IN SELECTED TISSUES OF NORMAL AND IRON-DEFICIENT RATS

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(Received January 18, 1995)

ABSTRACT: Iron and zinc distribution in selected tissues of normal and iron-deficient Sprague-Dawley rats were examined. Analysis of the data obtained showed that iron-deficiency anaemia resulted in a reduction (21.18% to 90.07%) of iron stores in the spleen, skeletal muscle, intestine, liver, diaphragm, kidney and stomach. Also, zinc stores were significantly reduced (11.30% to 89.00%) in the skeletal muscle, liver, spleen, stomach, small intestine, lungs, heart, diaphragm and kidney. However, there was resistance to iron depletion observed in the brain, heart and lungs of iron-deficient rats. The resistance to depletion of these trace mineral elements observed in these organs appears to be a compensatory device to preserve the functions of these organs in the face of severe iron-deficiency anaemia thus ensuring the survival of the organism.

Key Words: Trace mineral elements; Iron-deficiency anaemia; Iron and zinc distribution.

## INTRODUCTION

Iron is one of the most physiologically important trace elements in the tissue with its total content in the body varying with species, age, sex, nutrition and state of health (1). Although iron is present in very small amounts in the human body, it plays an important role in various metabolic functions while the major portion of it is used for the synthesis of haemoglobin and myoglobin. It also play an important role in the functioning of many other proteins and enzymes (2). Iron can also be stored in ferritin and haemosiderin depending on the previous iron nutritional status of the subject (1).

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The body of a normal adult male contains 4-5g of total iron (3,4) while a normal adult female contains about 2.5g of iron. The majority is found in red blood cells (about 1g of iron per kg of red blood cells) where it is incorporated into the haemoglobin molecule. Iron store, based on an indirect estimate, range between 0 and 1250 mg in adults, with menstruating women having an average store of approximately 300 mg (range 0-875 mg), menopausal women, 600 mg (range 0-1110 mg) and men 775 mg (range 240-1250 mg) (5). About 25% of the total body iron is stored in the reticuloendothelial system, liver, spleen, bone marrow, kidney and heart. In the muscle, iron (about 0.01 g/kg muscle) is stored in the form of myoglobin while the pancreas and the brain also store iron (6).

Zinc, like iron and some other elements, is an important micro-nutrient required for normal functional activities in animals and humans. The total amount of zinc in the human body has been estimated to be

between 2 and 3g and it is found in all human tissues in an amount varying from 10 - 20 mg/g wet weight of tissue (7). It is found in the red blood cells as a component of carbonic anhydrase. In whole blood, zinc is present in a concentration of about 900 mg/dl of blood while serum concentrations average 121 mg/dl where it is protein-bound (8). Its deficiency has been reportedly to result in growth retardation, hypogonadism and partial adrenal hypofunction. Taste and smell perception have also been found to be reduced in zinc deficiency (9).

Since reports on the distribution of these trace mineral elements (i.e. iron and zinc) in normal individuals are numerous, this paper attempts to evaluate their distribution in tissues in abnormal conditions such as iron-deficiency anaemia. Since low levels of zinc have been reportedly found in leukocytes in patients with chronic anaemia (10), a more comprehensive study of its distribution in other tissues in iron-deficiency is considered worthwhile.

## MATERIALS AND METHODS

*Animals*

Weanling rats of the Sprague-Dawley strain were used in this study. They were obtained from the Postgraduate Institute for Medical Research and Training, University College Hospital, Ibadan. All animals were fed with balanced diet and were apparently healthy before use.

*Induction of Iron-Deficiency Anaemia*

Animals were separated into three groups consisting of five rats per group. These are the experimental group (E) consisting of rats fed the iron-deficient basal diet (Table 1), the pair-fed (PF) control group which were fed an amount of iron-supplemented diet equal to that consumed the previous day by the corresponding experimental rats, and another control group which were fed the iron-supplemented diet *ad libitum*.

Table 1: Composition of iron-deficient diet[1].

| Ingredients | Amount (g/kg diet) |
|---|---|
| Casein | 850.66 |
| Vitamin-Gari Mixture[2] | 42.54 |
| Mineral Mixture[3] | 5.53 |
| Vegetable oil | 100.00 |
| Choline chloride | 1.27 |
| Fat-soluble vitamins[4] | see below |

1. To the control diet was added 300mg of $FeSO_4.7H_2O$ per kg diet.
2. Vitamin-Gari mixture contains the following in g per kg of preparation: thiamine-HCl, 0.20; riboflavin, 0.12; pyridoxine-HCl, 0.08; calcium pantothenate, 0.32; biotin, 0.04; choline chloride, 6.0; niacin, 0.50; folic acid, 0.10; vitamin $B_{12}$ (in mannitol), 0.0004; menadione, 0.66; gari, 991.64.
3. Mineral mixture contains the following in g/kg diet: $MnSO_4$, 1.25; $CuSO_4.5H_2O$, 0.04; NaF, 0.008; $ZnSO_4.7H_2O$, 0.02; $Na_2CO_3$, 0.60; $CoSO_4.7H_2O$, 0.004; KCl, 1.5; KI, 0.004; $MgSO_4$, 4.05.
4. Contains 5.0 mg vitamin A acetate and 375 mg $\alpha$-tocopherol in 50 ml vegetable oil given at 0.1 ml dose to each rat twice a week.

All the animals were placed on their respective synthetic diets for a period of eight weeks. During the period of feeding, they were housed individually in plastic cages and given deionised water *ad libitum* from plastic bottles fitted with stainless steel outlets. At the end of the experiment, the rats were killed by cervical dislocation.

*Haemoglobin Measurements*

To ascertain the state of iron deficiency, 8th week haemoglobin was determined by the cyanmethaemoglobin method (11).

*Removal and Digestion of Tissues*

At the end of the experiment, the rats were killed by cervical dislocation and the whole body was swabbed with 70% (v/v) ethanol. The rats were secured abdomen-

upwards on a dissecting board and rapidly dissected to remove the tissues needed. These tissues were thoroughly washed with normal saline solution to remove blood.

The digestion of tissues was carried out using a modification of the method described by Sadiq and Alam (12). In a typical assay, the tissues from each animal were weighed, minced and thoroughly washed again with normal saline to remove blood. The minced tissues (1g) were put in clean and labelled digestion tubes. Each tube received concentrated nitric acid (2 ml) and perchloric acid (1 ml). The bottles were left uncovered for 96 hours at room temperature to digest the tissues, then the digestion of tube content was carried out at 110$^{o}$C in a block digester for 4 hours. The tubes were cooled, 20 ml of deionized water was added and the digested tissues were filtered through Whatman No. 1 filter paper to remove undigested materials and gross fat. The volume of the filtrates was increased to 50 ml and stored at 4$^{o}$C before they were analysed for zinc and iron content.

*Determination of Iron and Zinc in Digested Tissues*

All analyses for iron and zinc in digested tissues were carried out using the atomic absorption spectrophotometer. All test samples were analysed in duplicates. Five determinations were taken on each sample and the standard deviation was computed. Results with more than 10% variation were rejected.

*Statistical Analysis*

The data obtained were subjected to statistical analysis. The differences between pairs of experimental (E) and pair-fed (PF) controls, between pair-fed (PF) and *ad libitum*-fed (C) controls and between experimental (E) and *ad libitum*-fed (C) controls were compared. The mean differences were subjected to Student's t-test for paired samples (13). Only P values less than 0.05 were considered statistically significant.

## RESULTS AND DISCUSSION

A summary of the eighth week mean haemoglobin levels of iron-deficient and control rats is presented in Table 2. The results indicate that after this period of feeding, haemoglobin level of iron-deficient rats reduced by 56.85% when compared with the pair-fed controls and by 54.82% when compared with the *ad libitum*-fed controls. This shows that at the end of eight weeks anaemia due to iron deficiency becomes apparent in the animals.

Table 2: Eighth week haemoglobin levels of iron-deficient and control rats.

| Groups | Hb Concentration (g/dl) |
|---|---|
| Iron-Deficient (E) | 6.80 ± 0.26 |
| Pair-Fed Control (PF) | 15.76 ± 0.30 |
| *Ad libitum*-fed Control (C) | 15.05 ± 0.25 |

Values represent the mean ± S.E. of five rats.

Iron-deficient animals exhibited poor body growth as shown in Fig. 1. When compared with the *ad libitum*-fed controls, the poor body growth can be attributed to loss of appetite as the animals belonging to this group turned from meal eaters to meal nibblers about the 14th day of feeding. The loss of appetite could have been induced by lack of dietary iron. This observation is supported by the fact that the pair-fed control group showed a comparable growth pattern with the iron-deficient group. The animals in the pair-fed group were allowed to eat an amount of diet equal to that consumed on the previous day by the corresponding experimental rats. On each occasion, the pair-fed rats finished their rations quickly, suggesting that they could have eaten more had they been fed *ad libitum*. Caloric restriction is thus responsible for the poor body growth observed in this group. The poor body growth exhibited by the iron-deficient rats may be a compensatory mechanism demonstrated by the iron-deficient animals essentially to

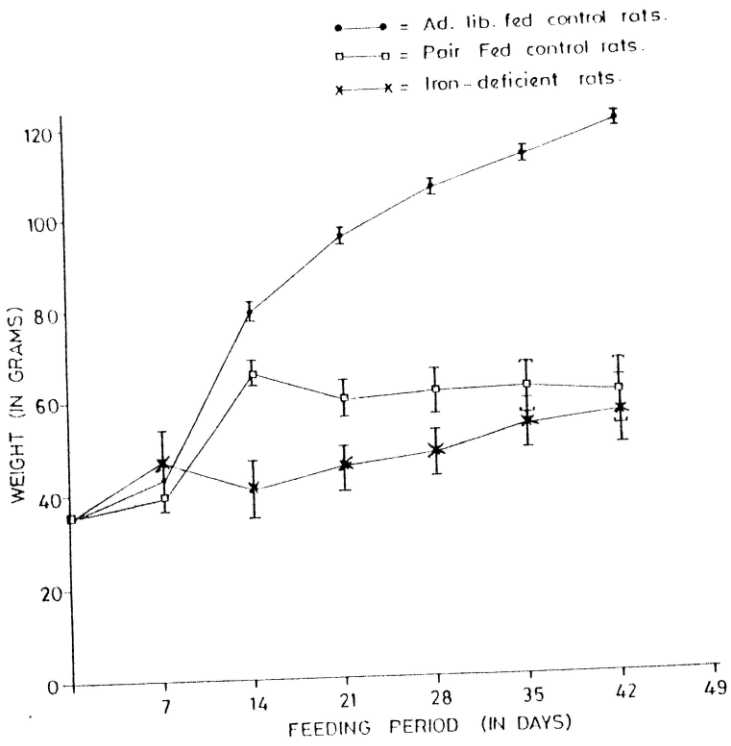


Fig. 1: Growth curve for iron-deficient ( ✖ ), pair-fed control ( □ ) and *ad libitum*-fed control ( ● ) rats. Each point represents the mean ± S.E. of five rats.

prevent further dilution of the body iron stores so as to keep the animal alive.

Table 3 summarizes the distribution of iron in the brain, diaphragm, heart, intestine, kidney, liver, lungs, skeletal muscle, spleen and stomach of normal and iron-deficient rats. The results show that in the tissues studied, there was no significant difference ($P > 0.05$) in the iron content of the brain, heart and lungs when the iron-deficient animals were compared with the pair-fed and the *ad libitum*-fed controls respectively. The resistance to iron depletion in these tissues may not be unconnected with the significance of the functions of these tissues to survival, thus making the resistance to iron depletion a compensatory change. The functions of the brain are central to all nervous control while the heart, in conjunction with the lungs, ensures free flow of blood and adequate delivery of nutrients and oxygen to the tissues.

The delivery of oxygen to the tissues is known to be impaired in iron deficiency as a result of the reduced haemoglobin concentration. Consequently, an increase in 2,3-diphosphoglycerate, a compound that alters the oxygen dissociation curve of haemoglobin in such a way as to increase oxygen delivery to the tissues has been reported (14,15). The increase in the intracellular levels of this compound is considered a compensatory change which is necessitated by the need to prevent extreme anoxia which could arise from decreased haemoglobin levels by efficiently ensuring adequate supplies of both glucose and oxygen to various peripheral tissues for oxidation. An increase in cardiac output at haemoglobin levels below 7 g/dl has also been reported (17). A resistance to iron depletion in the heart and brain is therefore considered another compensatory change in iron-deficiency anaemia to ensure normal functions of these tissues to enable the organism to survive when severely anaemic.

Reduction in iron stores were observed in the remaining tissues. The spleen appears to be the tissue where there was the highest depletion as no iron could be detected by the method used for iron assay. Since the spleen is a major storage compartment for iron and the iron stored in this compartment is utilized in times of need, a serious depletion of the iron store in the spleen is an indication of active erythropoiesis in iron deficiency anaemia. Since no iron comes through an exogenous source, a depletion results.

A very high rate of iron depletion in iron-deficiency anaemia was also observed in the skeletal muscle. When compared with the pair-fed and *ad libitum*-fed controls, a reduction of 89.98% and 90.07% respectively in iron stores was found. This high depletion may be due to the fact that the skeletal muscle is not considered as essential to survival as the heart, brain or lungs. Therefore, a depletion in muscles can be well accommodated without threatening the survival of the animal.

The intestine showed a reduction of 71.85% and 56.85% in iron stores in iron-deficient rats when compared with the pair-fed and *ad libitum*-fed controls respectively. This reduction may be due to the high turnover of the cells of the intestine where the iron content is not depleted as a result of withdrawal of iron from the diet of the iron-deficient rats. The liver, on the other hand, showed a reduction of 66.72% and 58.63% when the iron-deficient animals were compared to the pair-fed and the *ad libitum*-fed controls respectively. It is well known that the hepatocytes as well as the macrophages (Kupffer cells) of the liver, like the spleen, store iron which are made available on demand. Since erythropoiesis is not stopped in iron deficiency anaemia, the reduction in iron store observed in the liver of anaemic rats may be due to on-going erythropoiesis, even in severe iron deficiency. It appears, however, that the iron deposit in the spleen is first depleted before the liver is called upon. In all the tissues studied, there were no significant differences ($P > 0.05$) in the values obtained when the pair-fed and the *ad libitum*-fed controls were compared.

The diaphragm showed a reduction of 51.72% and 46.61% in their iron stores when the anaemic rats were compared with the pair-fed and *ad libitum*-fed controls respectively.

The kidney showed a reduction of 30.10% and 28.96% respectively when the

Table 3: Iron distribution in selected organs of iron-deficient, pair-fed control and *ad-libitum*-fed control rats

| Organ | Iron-Deficient Rats (E) (mg/kg wet wt.) | Pair-fed Control Rats (PF) (mg/kg wet wt.) | *Ad libitum*-fed Control Rats (C) (mg/kg wet wt.) | Statistical Comparison (P values) | | |
|---|---|---|---|---|---|---|
| | | | | E vs PF | E vs C | PF vs C |
| Brain | 12.26 ± 1.21 | 12.82 ± 0.26 | 13.86 ± 0.10 | N.S | N.S. | N.S. |
| Diaphragm | 20.10 ± 0.60 | 41.63 ± 0.71 | 37.65 ± 0.33 | < 0.05 | < 0.05 | N.S. |
| Heart | 41.28 ± 0.91 | 40.98 ± 0.25 | 39.89 ± 0.39 | N.S. | N.S. | N.S. |
| Small Intestine | 20.39 ± 0.31 | 72.44 ± 3.08 | 77.24 ± 3.85 | < 0.05 | < 0.05 | N.S. |
| Kidney | 40.89 ± 2.56 | 58.50 ± 1.60 | 57.56 ± 1.31 | < 0.05 | < 0.05 | N.S. |
| Liver | 51.94 ± 5.41 | 156.09 ± 9.51 | 125.56 ± 6.29 | < 0.05 | < 0.05 | N.S. |
| Lungs | 9.45 ± 0.12 | 9.83 ± 0.35 | 10.34 ± 0.27 | N.S. | N.S. | N.S. |
| Skeletal Muscle | 3.72 ± 0.06 | 37.14 ± 3.57 | 37.45 ± 2.43 | < 0.05 | < 0.05 | N.S. |
| Spleen | N.D.[3] | 98.79 ± 1.24 | 98.71 ± 1.21 | - | - | N.S. |
| Stomach | 16.56 ± 0.15 | 21.28 ± 1.03 | 45.43 ± 1.33 | < 0.05 | < 0.05 | < 0.05 |

Values represent the means ± S.E. of five rats. N.S. = Not significant. N.D. = Not detected.

same comparisons were made. The moderate reduction in the iron stores in the kidney of anaemic rats may also be a sparing mechanism fro this tissue to function effectively in severe iron deficiency. In one study (18), the weight of the kidneys per unit body weight has been found to be higher in iron-deficient rats, suggesting that these organs are very active in iron deficiency.

In the stomach, iron was reduced by 22.18% and 63.55% when the iron-deficient animals were compared with the pair-fed and *ad libitum*-fed controls respectively. Also, a 53.16% reduction was also observed when the pair-fed group was compared with the *ad libitum*-fed controls. This observation could be due to the fact that the pair-fed controls received less iron through the diet as a result of caloric restriction and the rate of turnover of the mucosa cells of the stomach may be comparable with the *ad libitum*-fed controls resulting in lower iron in the stomach of the pair-fed controls.

Table 4 summarises the effect of iron deficiency anaemia on zinc distribution in the same tissues studied for iron distribution. The results showed that the zinc contents of the brain in all groups were comparable. However, a reduction in zinc stores was observed in other tissues in the iron-deficient rats. When compared with the *ad libitum*-fed controls, there was 89.81%, 77.30%, 68.42%, 54.58% and 50.76% reduction in zinc stores in the skeletal muscle, liver, spleen, stomach and small intestine of anaemic rats respectively. A reduction of 89.00%, 77.76%, 69.31%, 24.77% and 51.88% was obtained for the skeletal muscle, liver, spleen, stomach and small intestine respectively when the results of the zinc stores of anaemic rats were compared with those of the pair-fed controls. No significant difference ($P > 0.05$) was obtained when the pair-fed controls and the *ad libitum*-fed controls were compared in all the tissues, except the stomach where a 39.63% reduction in zinc level was obtained.

The resistance to zinc depletion in the brain also appears to serve as a compensatory change. Zinc is an essential part of some catalytically active enzyme such as leukocyte alkaline phosphatase (19)., carbonic anhydrase (8) and carboxypeptidase (20). A disturbance in the metabolism of this element might lead to a disturbance in metabolic activities in which these enzymes are involved. A resistance to its depletion in the brain may, therefore, be a compensatory change needed to preserve proper organ functions.

The reduction in zinc levels observed in the skeletal muscle may be due to the fact that this organ could afford to lose its zinc without threatening the survival of the animals, while those observed for the liver, spleen, stomach and the small intestine may be as a result of the imbalance created in the zinc metalloprotein synthesis as a result of reduced enzyme activities that may accompany zinc depletion. the reduction obtained when the pair-fed controls were compared with the *ad libitum*-fed controls might be traceable to dietary sources. The mucosa cells of the stomach undergo rapid turnover. Caloric restriction reduces available dietary zinc thus reducing the zinc stores of the stomach

Other tissues where reductions in zinc stores were observed include the lungs, heart, diaphragm and kidney. When the results obtained were compared, 31.21%, 22.04%, 13.30% and 11.70% reduction was obtained in the lungs, heart, diaphragm and kidney respectively when the iron deficient animals were compared with the *ad libitum*-fed controls. Reductions of 31.54% and 13.62% were obtained for the lungs and kidneys respectively when the iron deficient rats were compared with the pair-fed controls. However, no significant differences ($P > 0.05$) were obtained for the heart and the diaphragm.

It appears from the results that the lungs, heart, diaphragm and kidneys show little resistance to zinc depletion in iron deficiency anaemia. These tissues also perform vital functions in the organisms, hence the need for a resistance to depletion to prevent further losses which might be detrimental to the animals.

Table 4: Zinc distribution in selected organs of iron-deficient, pair-fed control and *ad-libitum*-fed control rats.

| Organ | Iron-Deficient Rats (E) (mg/kg wet wt.) | Pair-fed Control Rats (PF) (mg/kg wet wt.) | *Ad libitum*-fed Control Rats (C) (mg/kg wet wt.) | Statistical Comparison (P values) | | |
|---|---|---|---|---|---|---|
| | | | | E vs PF | E vs C | PF vs C |
| Brain | 16.14 ± 0.10 | 15.80 ± 0.25 | 17.31 ± 0.21 | N.S. | N.S. | N.S. |
| Diaphragm | 11.76 ± 0.11 | 14.28 ± 0.59 | 13.56 ± 0.52 | < 0.05 | N.S. | N.S. |
| Heart | 12.70 ± 0.10 | 13.94 ± 0.38 | 16.29 ± 0.13 | < 0.05 | N.S. | < 0.05 |
| Small Intestine | 9.73 ± 0.47 | 20.22 ± 0.20 | 19.76 ± 0.12 | < 0.05 | < 0.05 | N.S. |
| Kidney | 22.26 ± 1.45 | 25.77 ± 1.21 | 25.21 ± 1.21 | < 0.05 | < 0.05 | N.S. |
| Liver | 8.67 ± 0.15 | 38.99 ± 1.02 | 38.19 ± 0.91 | < 0.05 | < 0.05 | N.S. |
| Lungs | 7.14 ± 0.09 | 10.43 ± 0.16 | 10.38 ± 0.04 | < 0.05 | < 0.05 | N.S. |
| Skeletal Muscle | 2.71 ± 0.05 | 24.64 ± 0.67 | 26.60 ± 0.13 | < 0.05 | < 0.05 | N.S. |
| Spleen | 10.14 ± 0.03 | 33.11 ± 0.55 | 32.11 ± 0.50 | < 0.05 | < 0.05 | N.S. |
| Stomach | 7.29 ± 0.12 | 9.69 ± 0.96 | 16.05 ± 0.24 | < 0.05 | < 0.05 | < 0.05 |

Values represent the means ± S.E. of five rats. N.S. = Not significant

ACKNOWLEDGEMENT: The authors wish to thank Dr. O. O. Oyedapo of the Department of Biochemistry, Obafemi Awolowo University, Ile-Ife, for assisting in getting the samples analyzed by atomic absorption spectrophotometry.

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