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Basic nutritional investigation

Preparation of iron-enriched baker's yeast and its efficiency in recovery of rats from dietary iron deficiency

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ABSTRACT

Objectives: Iron is an important mineral, essential for the health and function of mammalian cells. Despite its key role, iron deficiency in humans is common worldwide, often leading to significant health issues within the population. The aim of this study was to evaluate the potential of using iron-enriched baker's yeast as a source of iron, especially for the protection and recovery from conditions related to anemia.

Methods: Iron-enriched yeast was prepared by cultivating cells on basal medium comprising different iron concentrations. The effects of iron supplementation on animal health were assessed by feeding anemic rats with a variety of diets containing either inorganic iron or iron-enriched yeast. Body weight, iron bioavailability, blood parameters, and the activity of iron-containing enzymes (catalase) were studied.

Results: Iron accumulation in yeast cells increased with iron concentration, reaching a maximum of 15 mg/g when 32 mM iron was applied. Rat groups fed iron-enriched yeast had the highest feed efficiency, iron bioavailability, and hemoglobin concentration. The source of iron supplementation influenced catalase activity in kidney tissues, increasing from 70 U/g tissue in anemic rats to 90 U/g tissue (inorganic iron salt), 110 U/g tissue (inorganic iron salt and non-enriched dry yeast), 145 U/g tissue (iron-enriched yeast 15 mg/g iron) and 115 U/g tissue (iron-enriched yeast 30 mg/g iron). The histologic study of tissues from liver, kidney, heart, and spleen of rats from different groups showed that the damage observed in tissues of anemic rats, was not observed after feeding with iron-enriched yeasts.

Conclusion: The results demonstrated that ingestion of iron-enriched yeast is more efficient than inorganic treatment in recovery from iron deficiency, including tissue recovery in rats.

Introduction

Although iron is an abundant mineral, it has low bioavailability, and iron deficiency represents one of the most common nutritional deficiencies worldwide, with significant implications to public health [1]. The average adult male (70 kg) has a total body iron content of approximately 4 g, which remains relatively constant throughout adult life [2]. This is maintained by balancing loss of iron with assimilation via nutritional intake. Persistent deviation in iron intake can result in either iron deficiency, or iron overload (hemosiderosis and hemochromatosis) [2]. The most obvious manifestation of iron deficiency is anemia, but lack of iron can also have an adverse effect on the immune system [3,4] and cognitive development [5,6]. Iron is an essential nutrient because iron cofactors, including heme and iron-sulfur clusters, are required for the activity of a number of enzymes involved in a range of cellular processes. Furthermore, iron is also an important component of molecules that undergo redox reactions in cells [7,8]. Anemia and iron deficiency may cause serious diseases; for example, they are quite prevalent in patients with heart failure. In the past few years, there has been an enormous interest in the subject of iron deficiency and its management in patients with heart failure [9].

Because leavened bread, produced using *Saccharomyces cerevisiae* (baker's yeast), is a staple component of diets in many areas of the world, the potential for using yeast biomass enriched

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with iron as a feed supplement has the potential to deliver a safe and efficient mechanism for the prevention or recovery from anemia. Previous studies have shown that iron bound to organic carriers, such as cytochrome C, catalase, and other proteins and enzymes in yeast cells, are more easily absorbed and have a reduced potential for toxicity [10]. Additionally, yeast biomass is known to be a good source of protein, amino acids, and vitamins, and has been shown to have a positive nutritional effect in animals and humans [10]. Despite the suitability of yeast for nutrient transfer, the uptake, storage, and utilization of iron in *Saccharomyces cerevisiae* cells is tightly regulated. In particular, the metal uptake and assimilation processes are complex and dependent on the chemistry of metal ions, specific surface properties of the organism, cell physiology, and the physicochemical influence of the environment [10]. Consequently, in this study the capacity of a baker's yeast strain to assimilate iron was determined, and the resulting iron-enriched baker's yeast was evaluated for its potential in alleviating symptoms of iron deficiency in Wistar rats.

Materials and methods

Preparation of iron-enriched yeast

A commercial baker's yeast strain was obtained and isolated from the local market (Aleppo, Syria). Yeast propagation was conducted in 250-mL Erlenmeyer flask containing 100 mL basal medium (20 g/L glucose, 0.5 g/L yeast extract, 5 g/L ammonium sulfate, 1 g/L potassium dihydrogen phosphate, 0.5 g/L magnesium sulfate, pH adjusted to 5.2 with 5 N NaOH). Yeast cultures were enriched with iron by supplementation with ammonium iron (III) citrate in concentrations of 0.5%, 1%, 2%, and 4% (W/V) corresponding to 16, 32, 64, and 128 mM iron. The cultures were incubated at 30°C for 24 h, with shaking at 150*g*. In each instance, the entire cell culture was recovered by centrifugation, excess liquid was decanted, and samples were desiccated using a drying oven (Memmert, Egypt). Dried yeast samples were collected and used for further analysis (iron content and as a nutritional supplement) as described below.

Evaluation of iron content in iron-enriched yeast

After 24 h, cultivation cultures were centrifuged (5 min, 4000*g*) and the supernatant discarded. The pellet was washed three times with distilled water to remove loosely associated fraction of iron from yeast cell surface. Washed yeast biomass was dried at 105°C until a constant mass was achieved, and digested by adding 1 mL of 65% (v/v) nitric acid to 20 mg dry biomass, followed by heating for 30 min at 140°C. After cooling, samples were diluted with distilled water to a final volume of 5 mL and the content of iron was analyzed by Flame Atomic Absorption Spectrometry in the Agricultural Research Center, Giza, Egypt. The amount of total iron was calculated per gram of dry weight (mg iron/g dry wt.) as described previously [10].

Animals and diets

Forty-eight newly weaned male and female Wistar rats (initial body weight, 75–85 g) were obtained and housed individually in stainless steel, wire mesh-bottom cages. A 12-h light/dark cycle and an environmental temperature of 25°C ± 2°C were maintained. Newly weaned rats were chosen, because individuals of this age are similar regardless of sex; physiological, hormonal, or metabolic changes are not observed between males and females at this early stage of life. This was verified in-house by analysis of data, which showed that there was no difference between young rats based on sex distinction (data not shown). Rats were randomly assigned to one of six dietary groups (eight rats per group) as illustrated in Figure 1. The control group was fed a basal diet containing 30 mg/kg of iron for 56 d (total experiment time). Precise composition of the basal diet used was based a previous study [11] and shown below.

Iron deficiency was induced as described previously [12] with minor modifications: A low iron basal diet (3 mg/kg) was provided in conjunction with bleeding of 1 to 2 mL of blood every week for 4 wk until the iron and hemoglobin reached a designated low level (109–112 g/L hemoglobin and 3 mg body iron). These anemic rats were assigned to five groups of eight rats (four males and four females). Groups were examined by feeding one of the following diets for an additional 28 d: group 1 was fed a basal iron-deficient diet (no iron added). Group 2 was fed a basal iron-deficient diet plus ferrous sulfate heptahydrate (30 mg/kg iron diet) group 3 was fed a basal iron-deficient diet plus ferrous sulfate heptahydrate (30 mg/kg iron diet) plus 1 g/kg of nonenriched yeast diet. Group 4 was fed a basal iron-deficient diet plus iron-enriched yeast (containing 15 mg/kg iron diet). Group 5 was fed a basal iron-deficient diet plus iron-enriched yeast (containing 30 mg/kg iron diet). Total food intake was determined at the end of the experiment; it should be noted that in each instance, all animals were marginally underfed to ensure that each ingested the total quantity of food provided.

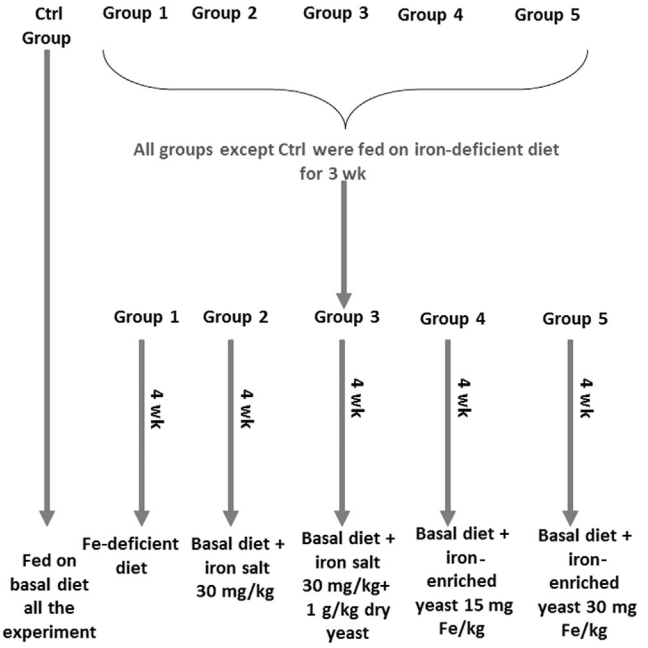

Fig. 1. Design of rat feeding experiments.

The body weight of rats was measured every week throughout the 56 d of dietary treatment. After 14, 21, 28, 42, and 56 d of the dietary program, blood (0.5 mL) was collected from the rats' eyes into heparinized micro-capillary tubes.

The basal diet was prepared as follows: skim milk (50% casein), 400 g/kg diet; cornstarch, 603 g/kg; vegetable oil, 80 g/kg; sucrose, 50 g/kg; mineral mix, 40 g/kg; vitamin mix, 10 g/kg.

The mixtures of minerals and vitamins were prepared according to a previously described method [11] as follows:

Vitamin mix contained the following (g/kg mix): inositol, 25; ascorbic acid, 5; calcium pantothenate, 0.67; thiamine hydrochloride, 0.27; pyridoxine hydrochloride, 0.53; nicotinic acid, 1; menadione, 0.25; riboflavin, 0.27; p-amino benzoic acid, 0.50; folic acid, 0.067; biotin, 0.26; all-rac-α-tocopheryl acetate, 3.60; retinyl palmitate, 0.034; cholecalciferol, 0.21; vitamin B_{12} 1 mannitol, 3.33; choline chloride (70% solution), 71.50; and cerelose, 887.8.

Mineral mix contained the following (g/kg mix): CaCO3, 139.7; CaHPO4, 166.6; K2 HPO4, 133.6; NaCl, 21.2; MgSO4, 49.5; ZnCO3, 0.8; MnSO4.H2O, 0.61; CuSO4.5H2O, 0.66; KI, 0.0033; CrK(SO4).12H2O, 0.048; Na2SeO3, 0.015; Na2MoO4.2H2O, 0.0063; and cerelose, 481.1.

Blood analysis

Hematocrit was measured by centrifugation of blood and expressed as the volume percentage of red blood cells (RBCs). Hemoglobin concentration was measured using a hemoglobin determination kit (Biodiagnostic Inc., Egypt). Serum iron concentration and total iron-binding capacity (TIBC) were measured using iron and TIBC kit (Biodiagnostic Inc., Egypt). Transferrin saturation (TS) was calculated from the ratio of serum iron to TIBC.

Catalase assay

Catalase activity was determined in plasma and kidney tissue after homogenate preparation by tissue perfusion with a phosphate-buffered saline solution, pH 7.4 containing 0.16 mg/mL heparin to remove any RBCs and clots before dissection. Tissue was homogenized in 5 to 10 mL cold buffer (50 mM potassium phosphate, pH 7.5. 1 mM EDTA)/g of tissue; the homogenate was then centrifuged at 10,000*g* for 15 min at 4°C. The supernatant was removed and stored on ice. If the assay was not conducted on the same day, the sample was frozen at −80°C.