Nutritional Yeast Ferritin-Iron Complex:  
A Novel Source of Dietary Iron

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Received October 08, 2021; Revised November 10, 2021; Accepted November 23, 2021

Abstract Iron deficiency anemia (IDA) is the leading nutritional disorder in the world. Iron deficiency (ID) occurs commonly in the US, particularly among children and women and in the elderly. Its impact on quality of life includes decreased cognitive ability and increased fatigue. Thus, there is clearly a need for effective and economical approaches to providing adequate dietary iron. We introduce nutritional yeast (S. cerevisiae) technologically modified to express human H-ferritin as a potential source of dietary iron. The efficacy of the yeast ferritin complex (YFC) was tested in established rat and monkey models of ID. In the rat model of ID, YFC improved hemoglobin (Hgb), hematocrit (Hct) levels as well as plasma iron and transferrin saturation and liver and spleen iron concentrations. Moreover, the YFC achieved a larger effect on Hgb and Hct with consumption of less iron compared to ferrous sulfate over a similar period. In the monkeys, YFC supplemented diet significantly improved Hgb levels within one month and increased mean corpuscular volume (MCV) by two months. To demonstrate that the Fe in the YFC was utilized by RBCs, we used a labeled isotope approach and found Fe from the ferritin in the monkeys’ RBCs with 5 days of consumption. This is the first attempt to use H-ferritin homopolymer as a nutritional iron source. Previous data from our laboratory and others have established H-ferritin as a significant iron delivery protein to the brain. Thus, H-ferritin appears to have a novel function as an iron delivery protein independent from its cooperation with L-ferritin to form an iron storage protein.

Keywords: iron deficiency, nutritional yeast, ferritin, iron

Cite This Article: James R. Connor, Erica L. Unger, Ralph L. Keil, John Flanagan, Stephanie M. Patton, Gabriele R Lubach, Martin M. Schafer, and Christopher L Coe, “Nutritional Yeast Ferritin-Iron Complex: A Novel Source of Dietary Iron.” American Journal of Food and Nutrition, vol. 9, no. 3 (2021): 122-131.
doi: 10.12691/ajfn-9-3-5.

1. Introduction

Iron deficiency is estimated by the World Health Organization to affect 3-4 billion people worldwide; placing it as the top nutritional disorder in the world [1]. It is the primary cause of anemia and has an estimated worldwide economic impact in the billions of dollars. Infants and women of child-bearing age are most affected by iron deficiency anemia (IDA), with a higher prevalence in individuals of lower socioeconomic status [2]. Infants are of particular concern because IDA during early life has been associated with developmental delays and poorer cognition, motor performance and social-affective behavior [3,4,5,6]. Using rodent models of ID and IDA, these developmental and behavioral outcomes have been linked to changes in neurotransmitter signaling [7], myelination [8], and hippocampal neuron architecture [9]. Other groups with high prevalence of ID include postpartum women [10] and post-surgical gastric bypass patients [11]. While iron supplementation is required in all of the aforementioned groups to minimize the effects on development and/or health, the individuals at highest risk of iron deficiency (low income women and children) are often least likely to consume supplements containing iron [12].

There are several currently accepted methods of iron supplementation, including oral iron tablets and intravenous (IV) iron, with each having known limitations or drawbacks. Oral iron supplements, including ferrous sulfate, ferrous gluconate, ferric citrate and ferric sulfate, have limited efficacy due to poor absorption. Moreover, gastrointestinal side effects are common, which leads to reduced compliance [13]. Intravenous (IV) iron treatments are frequently successful at rapid resolution of anemia but are associated with significant risks and are expensive.
Because of the interest and need for alternative dietary iron sources, plant-based approaches have been developed. Plant breeding strategies have led to the cultivation of biofortified staple food crops to improve the nutritional status of populations who are looking beyond meat as an iron source or who are most at-risk for ID. The efficacy of iron-biofortified rice, beans and millet have all been investigated in iron-deficient populations [15,16,17]. The results of these studies have been inconsistent, with some studies showing that iron-fortified food crops improve iron status [15,16,17], and others showing no improvement [18]. Importantly, studies that used genetically modified rice to over-express plant ferritin found no enhancement in iron accumulation in rice seeds between transgenic and non-transgenic lines and also showed iron absorption at rates no better than chemical compounds [19,20]. In this study, we chose to address the challenge of finding an efficient and economical source of dietary iron by using nutritional yeast that has been biotechnologically modified to express H-ferritin. The modification to express H-ferritin significantly increases the ability of the yeast to accumulate iron. Yeast is a dietary source of protein in many cultures and therefore can be readily introduced as a dietary supplement.

2. Materials and Methods

2.1. Construction of the YFC Strain

An approximate 600bp BamHI-Xhol fragment containing the human Fth1 gene was cloned into pENTR1a (ThermoFisher) cut with these same enzymes to form plasmid pLS652. Gateway® technology was used to transfer this FTH1 gene into pAG426GPD-ccdB, a yeast constitutive expression vector with the strong promoter from the TDH3 gene (also called the GDP promoter), which encodes glyceraldehyde 3-phosphate dehydrogenase, and the terminator sequence from the CYC1 gene, which encodes cytochrome c isoform 1, to form pLS649. The S.cerevisiae URA3 gene in pLS649 was mutated by cutting at the unique Stul site in this gene and inserting an 8-bp linker containing Xhol recognition sequence to make pLS655. This plasmid was cleaved at its unique EagI site just downstream of the CYC1 terminator and the 1.6 kb EagI fragment containing the URA3 gene from K.lactis was inserted into create pLS659. PCR primers upstream of the TDH3 promoter and downstream of the K.lactis URA3 gene were used to amplify the TDH3:FTH1 expression cassette linked to URA4. This fragment was transformed into yeast strain BY4741 MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0. The linear PCR product ligated in yeast to form a closed circular product that then inserted into the yeast chromosome by homologous recombination at the TDH3 promoter to produce strain P3190.

2.2. Yeast Ferritin Complex Preparation and Analysis

P3190 was grown in synthetic complete (SC) medium lacking uracil [26] with 6mM FeSO4 at 30°C with shaking for two days. Cells were collected by centrifugation and washed once with sterile water. Following resuspension, cells were incubated at 65°C for 30 min to pasteurize. Cells were then washed two additional times with sterile water to remove unincorporated iron and lyophilized.

Yeast expressed human heavy chain ferritin was isolated from lyophilized P3190 cells. The cells were washed 2x with 20ml/gm cells of buffer containing 10mM Tris-HCl pH 7.5 and 2mM EDTA and the final pellet resuspended with a dounce homogenizer in a buffer containing 25mM Tris-HCl pH 7.5 and 150mM NaCl (10mL/gm original cell pellet). The cells were lysed by 5 passes through a M110P microfluidizer (Microfluidics, Westwood MA) cooled to 4°C and processed at 30000 psi and the cell debris was removed by centrifugation for 30min at 21kxg and 4°C. The supernatant was incubated at 70°C for 10min and the precipitated protein removed by centrifugation at 21kxg and 4°C. Ferritin was precipitated from the supernatant by addition of solid AmSO4 to 60% final concentration and incubation with stirring at room temperature for 30min. The precipitate was collected by centrifugation at 21kxg and 4°C and resuspended in 50mL of 25mM Tris-HCl pH 7.5 (Buffer A) and then dialyzed overnight against this buffer. The dialyzed protein was centrifuged at 21kxg to remove any precipitated protein and the supernatant was then loaded onto a 5mL HiTrap Q HP (Cytiva) anion exchange column equilibrated in Buffer A. The unbound and weakly bound proteins were eliminated by washing the column with 5 column volume Buffer A + 50mM NaCl and the bound protein eluted in 10 column volume linear gradient to Buffer A + 500mM NaCl. The fractions containing ferritin were identified by coomassie stained SDS-PAGE and by their absorbance at 310nm. The peak fractions were pooled and concentrated using a 100k molecular weight cutoff amincon-15 ultra centrifugal filter (Millipore) and then further purified by chromatography, in 200μL aliquots on a Superose 6 Increase column (Cytiva, USA) equilibrated in SEC Buffer (25mM TrisHCl pH 7.5; 150mM NaCl) and the protein eluted in this buffer. The fractions from this column were analyzed to determine the protein purity by coomassie stained SDS-PAGE, protein concentration using a Bradford assay (BioRad, CA) with BSA as the standard and iron content using a ferrozine-based assay [21]. The peak fractions were pooled and concentrated down to ~0.5mg/mL based upon the concentration determined using the Bradford assay on the final concentrated fraction, frozen by snap-freezing in liquid N2 and stored at -80°C.

2.3. Electron Microscopic Analysis of Heavy Chain Ferritin Isolated from P3190

Formvar-coated carbon grids (300 μm mesh; EMS, PA) were prepared by plasma cleaning (air) for 60 s using an Emitech K590X plasma cleaner (Diener Electronics, Germany) immediately prior to use. A 4-μl aliquot of the purified ferritin (100μg/mL in SEC buffer) was applied to the grids and incubated at room temperature for 60s. Excess protein solution was removed from the edge of the grid using thin strips of Whatman No. 1 paper. The grids were washed 3 times with distilled water by placing 35-μl drop and each time touching the surface with filter paper and excess water removed as before. For unstained specimens the grids were then allowed to air dry for ~1hr at room temperature and the data collected immediately.
2.4. Rodent Feeding Studies

Twenty day old male Sprague Dawley rats (Envigo) were housed 1 per cage in hanging wire cages in a temperature (23 ± 2°C) and humidity (40%) controlled room maintained on a 12:12 hr light/dark cycle (lights on 0600 to 1800). Rats were fed an iron deficient diet (ID; 2 ppm iron) or supplement diets ad libitum as indicated. The ID diet was prepared following the recipe of the American Institute of Nutrition (AIN)-93G diet with cornstarch as the sole source of carbohydrate and without the addition of iron. Iron levels for all diets were verified using atomic absorption spectrometry after wet digestion with nitric acid (Perkin Elmer). All experimental protocols were in accordance with The National Institutes of Health Animal Care and Use Committee.

In the first study, rats were fed an ID diet for 26 days (P21 – P47), which produced anemia (mean hematocrit and hemoglobin levels were 5.3 ± 0.2 and 16.2 ± 0.1%, respectively). At P47, rats were then divided into 4 dietary groups (n=3-5/group) balanced by body weight: 1) ID diet (2 μg iron/g diet; n=4), 2) ferrous sulfate supplemented diet (50 μg iron/g diet; n=3), 3) YFC diet (32 μg iron/g diet; n=5), 4) yeast diet without additional iron or H-ferritin (2 μg iron/g diet; n=5). Rats were fed the assigned diet for 14 days, and food consumption was monitored throughout the study. The YFC and ferrous sulfate supplemented diets were prepared using the ID diet (2 ppm iron) as the base.

In a subsequent study, iron absorption was determined for the YFC and ferrous sulfate diets. Sprague Dawley rats were housed as above and fed an ID diet for 16 days, producing anemia with a mean hematocrit and hemoglobin level of 6.04 ± 0.1 and 22.0 ± 0.4%, respectively. Rats were then divided into four groups balanced for weight (n = 4-5 per group) and fed either a YFC or ferrous sulfate diet at one of two iron concentrations (17.5 μg iron/g diet or 35 μg iron/g diet) for 10 days. Feces were collected and weighed, and food consumption was measured to the nearest 0.1 g throughout the study for each diet group. Iron levels in the feces and diet were determined by atomic absorption spectrometry. Iron absorption was then determined by: ((total μg of iron consumed – total μg of iron excreted)/ total μg of iron consumed)*100.

2.5. Hematology

Blood (30 μL) was collected by tail venipuncture from each rat every 3-5 days throughout the study and at sacrifice to monitor Hb and hematocrit (Hct) levels. Hb values were determined photometrically using cyanomethemoglobin standard solution and according to the manufacturer’s instructions (Sigma Aldrich, St. Louis, MO). Hct levels were determined after centrifugation of whole blood in heparinized microcapillary tubes (13,700 x g, RT, 5 min).

At P61 (study 1) or P47 (study 2), rats were weighed, decapitated after brief exposure to CO2, and trunk blood was collected. Whole blood was centrifuged (16,110 x g, 4°C, 15 min) and then sera were stored at -80°C. Liver and spleen were rapidly removed from each rat, weighed, and stored at -80°C. Serum iron, total iron binding capacity (TIBC), and liver and spleen non-heme iron were determined using standard methods. Transferrin saturation (%) was calculated using serum iron and TIBC data.

2.6. Statistical Analysis for Rodent Feeding Studies

Rodent hematology and iron data were analyzed by one-way ANOVA with diet as the between-subjects variable (Systat 13.2, Richmond, CA). Pair-wise comparisons between control and the other treatments were made using the Tukey’s t-test. Data showing change in Hb and Hct per mg iron consumed were analyzed by t-test. P-values were set at p<0.05.

2.7. Monkey Studies

As described previously, a standardized model of infantile anemia was generated in monkeys by providing a commercial diet with only moderate levels of iron (225 mg/kg) to adult female rhesus monkeys in a large established breeding colony. This diet was consumed during both the pregnancy and lactation periods (PMI 5L1Q). With this husbandry protocol, between 20-30% of the infants develop a growth-related iron deficiency by the end of the nursing phase. Their iron status at 6-7 months of age was screened with hematology panels to identify subjects for two experiments. Criterion for ID included a Hb below 10 g/dL, and a mean corpuscular volume (MCV) below 60 fL.

Eleven ID monkeys were tested in the iron absorption experiment using yeast containing a stable iron isotope, 57Fe. Five consumed one dose at 6 mg/kg body weight, and blood was collected on the following day or after one week (Days 2 and 7). Six were given 5 daily doses (M-F), and blood collected on days 2 and 7 (N = 3 and 6, respectively).

Thirty-one ID monkeys were evaluated in an additional and more extended treatment experiment. Eighteen monkeys (14 M, 4 F) were given daily oral supplements of the standard YFC at 6 mg/kg body weight for either one or two months (N = 11 and 7 respectively). Thirteen different ID monkeys (9 M, 4 F) served as untreated controls. Their hematology was assessed at 6 and 8 months of age to examine if there were age-related changes across a 2-month period that was comparable to the treatment period.

2.7.1. Hematology

Complete Blood Counts (CBC) including Hb and MCV were determined at a local CLIA certified clinical laboratory familiar with monkey blood (Meriter Labs, Madison WI). It had been shown previously that the anemic criteria of Hb <10 mg/L and MCV < 60 fL were
strongly associated with low serum ferritin and TSAT in infant rhesus monkeys [22,23].

2.7.2. 57Fe uptake

The yeast growth conditions used for the stable isotope study in monkeys were identical to those used for the rodent study described above with the exception that the 1 M FeSO4 · 7H2O stock was used to enrich the SC-uracil media, consisting of a 50:50 ratio of 57FeSO4 (Isoplex, USA, San Francisco, CA) and 56FeSO4 (Sigma Aldrich, St.Louis, MO). Following lyophilization, the yeast was subjected to ICP-MS analysis to determine exact $^{57}/^{56}$Fe ratio and total iron concentration and these values were used to calculate the volume of yeast needed for daily dosing at 6 mg/kg body weight. As described above, 11 ID monkeys were given YFC orally for either one day or 5 days (N = 5 and 6, respectively) and blood collected either on the morning after the first dose or at one week after dosing started, two days after the last yeast had been consumed. Gut absorption of the $^{57}$Fe and incorporation into RBC was determined by evaluating the enrichment of $^{57}$Fe relative to $^{56}$Fe using ICP-MS.

2.7.3. YFC Treatment

The value of a more sustained oral treatment with the standard YFC was evaluated for 1-2 months. Growing infant monkeys were screened at 6-7 months of age to identify potential subjects meeting criteria (Hb below 10 mg/dL and MCV between 50-60 fL). Eighteen (14M, 4F) infant monkeys were screened at 6-7 months of age to identify potential subjects meeting criteria (Hb below 10 mg/dL and MCV between 50-60 fL). Eighteen (14M, 4F) infant rhesus monkeys [22,23].

2.7.4. ICP-MS Methods

Digestion-

Whole blood (60 or 100 µL) added into an acid-cleaned 15 mL polypropylene centrifuge tube. Digestion acids (1.00 mL ultra-high purity HNO3 and 0.250 mL ultra-high purity HCl (Optima® grade)) gently mixed with the blood and pre-digested for 1 hr at room temperature. High-purity, stabilizer-free, hydrogen peroxide (H2O2) is then added to the tube in three 0.250 mL increments (0.750 mL total). Digestion tubes incubated at 85 °C for 16 hr. After cooling, the digested blood is diluted to final volume of 10.0 mL with high-purity Type 1 water (MQ). Quality control (QC) samples included in this digestion step are 3 reagent blanks and 2-3 sample duplicates per batch.

Magnetic-sector ICP-MS (SF-ICPMS) Analysis of Total Iron and Iron Stable Isotope Ratios-

Digested samples were pre-screened for total Fe concentration by dilution with 2% v/v HNO3 and SF-ICPMS analysis. Samples are then diluted 5-10fold with 2% (v/v) HNO3 to achieve a target ion count rate of 200,000 cps of $^{57}$Fe for high-precision isotope ratio analysis. All data acquired in medium resolution (R~4000) with all spectral interferences on the quantified isotopes are mass-resolved. The iron stable isotope ratio analysis was performed on a Thermo-Fisher Element2 XR - double focusing, high resolution magnetic-sector ICPMS, configured with platinum skimmer and sampler cones, a PFA low-flow (0.4 ml/min) nebulizer, Quartz torch with a 1.5mm injector, and ESI PC3-FAST autosampler with a 2.5mL loop. The plasma was operated at an RF Power of 1160 W with argon gas flows of 16.0 L/min (cool); 0.88 L/min (aux); and 0.968 L/min (sample-neb).

Samples were analyzed in pairs, bracketed by check standards (High Purity Standards Iron at 500 µg/L) in a modified standard-sample-standard data-acquisition approach. Each sample analysis consisted of 10 separate runs of 18 passes and the reported $^{57}$Fe/$^{56}$Fe ratio was determined from the average ion count ratios of 10 runs. A reference material with certified $^{57}$Fe/$^{56}$Fe ratio (CRM - IRMM-014) is run with each analytical sequence.

3. Results

To characterize the human heavy chain ferritin (Fth1) expressed in the yeast cell line grown in high iron containing media, we purified the protein using standard biochemical and chromatographic methods. All of the Fth1 protein was found in the soluble fraction after cell lysis and was purified to >90% purity using a combination of heat treatment, ammonium sulfate precipitation, cation exchange and size exclusion chromatography with a yield of ~1mg of purified Fth1 per gm wet weight of cells. The majority of Fth1 (Figure 1A, B) eluted from the SEC column in a volume consistent with that expected for a globular protein of mass ~500kDa. In addition, a fraction of the Fth1 eluted earlier in a volume consistent with the Mw of a globular >800kDa protein. The vast majority of the protein in these two species were Fth1 (Figure 1C) as seen in the coomassie stained gel of fractions taken through the peaks indicating that it assembled into large, defined, oligomeric complexes expected for the native Fth1 24-mer and possibly a dimer of this complex. Both Fth1 species contained significant amounts of iron, based upon the ferrozine assay, and, due to the purity of these bands, we estimate that, on average, there were ~110 iron atoms per ferritin monomer. The purified peak fractions from the SEC column was characterized by electron microscopy of negatively stained and unstained protein (Figure 2A, B). In the negatively stained images circular, stain excluding (white), particles of ~12nm with a central electron denser core were routinely observed. These particles have the size and properties expected for Fth1 as a 24-mer. In the unstained images, circular, 4-8nm electron dense particles were observed. Close inspection of these particles suggest that they are not significantly mineralized, as no evidence of fine structure was observed either in real or reciprocal space. Further, the observed closest approach between adjacent particles is also consistent with the presence of an unobserved protein shell preventing the particles from touching. Taken together these data indicate Fth1 produced in yeast form native oligomers containing non-mineralized iron rich cores. Moreover, the data are consistent with an average iron content of the 24-mer of ~2500 iron atoms per complex in this preparation.
Figure 1. YFC expresses H-ferritin containing iron. Panel 1a is an elution profile of human heavy chain ferritin purified from yeast. The column was eluted in SEC buffer and the absorbance at 260, 280 and 310nm and the conductivity was monitored. The absorbance at 280nm and conductivity are shown. Due to the high absorbance of the iron the absorbance at the wavelength of the main peak eluting at ~14mL was saturated at its peak with this sample load. The totally excluded volume is shown by the conductivity trace (grey) and results from a slight mismatch of the salt concentration in concentrated protein sample and elution buffer. Panel 1b shows that iron co-elutes with the ferritin oligomer. The estimated Fth1 oligomeric concentrations (510kDa) estimated from a Bradford assay (black squares) and iron concentration (red squares) as function of elution volume is shown. The iron content of the total excluded peak was at baseline levels (data not shown). Panel 1c shows that Fth1 is the dominant species eluting in the Superose 6 chromatogram. Coomassie stained 12% SDS-PAGE gel. Lane 1 is the sample prior to loading on the column. Lanes 2-14 are 5μL from every other faction through peaks observed in the chromatogram. Lane 15 is the molecular weight standards. A line migrating at a Mw of ~39kD is observed throughout this gel and is an artefact of unknown origins.

Figure 2. Electron Micrographs of H-ferritin from YFC. (A) Yeast expressed Fth1 assembles into a ~12nm diameter stain excluding particles. The ferritin protein is observed as stain excluding (white density). In many of the particles a central darker region consisting of the hollow or iron filled core can be seen in these images. (B) The central cores of unstained Fth1 are dense particles of 4-8nm in electron microscopic images. Grids of unstained Fth1 reveal a large number of black (electron rich) particles consistent with the iron cores of ferritin. The size and density of these particles suggest some expected variability in the actual iron content of the particles. The variability could either be due to an actual difference in iron loading in the yeast, or could be due to loss of iron from some particles either during purification or grid preparation.
3.1. Rat Dietary Studies

**Hematology**

Rats fed YFC and ferrous sulfate diets had improved Hgb (Figure 3A) and Hct levels (Figure 3B) as compared to ID rats at 7, 9 and 14 days after starting the supplemented diets (p<0.01). The Hgb and Hct increase in rats receiving the YFC diet (32 ppm) was larger on days 9 and 14 (p<0.05) than observed with the ferrous sulfate diet (50 ppm). Hgb and Hct levels in rats fed yeast without ferritin and iron were similar to ID rats throughout the supplementation period.

At the end of the 14-day treatment, only rats fed YFC had significantly higher plasma iron (Figure 4A; p<0.05) and transferrin saturation (Figure 4B; p<0.05) when compared to ID rats. Liver iron (Figure 4C; p<0.05) and spleen iron (Figure 4D, p<0.05) were higher in YFC rats compared to rats receiving yeast without ferritin and iron. Yeast alone without ferritin or iron supplemented were associated with similarly low levels of plasma iron and transferrin saturation comparable to ID rats.

Diet consumption was determined throughout the experiment. Rats fed the YFC diet or the ferrous sulfate diet consumed more food than rats receiving the ID diet or the control yeast diet. On average, the amount of food consumed per day was 13.9 g for the YFC diet, 13.7 g for the ferrous sulfate diet, 10.1 g for the ID diet, and 10.4 g for the yeast without H-ferritin or iron supplemented (P<0.01). When the increments in Hgb and Hct were reanalyzed with respect to the mg of iron consumed, the statistical analyses indicated that YFC was more efficient than ferrous sulfate (Figure 5). Specifically, YFC produced a significantly greater change in Hgb (p<0.001) and Hct (p<0.01) per mg of iron consumed than the ferrous sulfate diet.

A second feeding study was performed to compare the relative iron absorption rates of the YFC and ferrous sulfate diets. The iron in YFC diet appeared more efficiently absorbed (almost 56.7 ± 1.9%) at a lower concentration compared to ferrous sulfate (43.4 ± 5.7%) although this difference was not statistically significant (Figure 6). At a higher concentration, iron absorbed from the YFC and ferrous sulfate diets were similar: at 49.1 ± 2.4% and 50.7 ± 14.5%. In the latter case, ferrous iron absorption increased with higher doses of iron, whereas iron from the YFC decreased, suggesting that there is a regulation to limit iron uptake from the yeast that can be explored in future studies.

![Figure 3. YFC is more effective at correcting Hgb (A) and hematocrit (B) levels than ferrous sulfate.](image-url)
Figure 4. YFC more effectively corrects iron deficiency measures than ferrous sulfate. Plasma iron levels (A); transferrin saturation levels (B); liver iron (C); and spleen iron (D). * p<0.05; **p<0.01 compared to ID diet. * Different from ID; † different from Yeast-No Iron/ferritin

Figure 5. YFC is more efficient at improving Hemoglobin (Hb) and hematocrit (Hct) than ferrous sulfate. Diet consumption was similar for rats fed YFC or the ferrous sulfate diet but the improvement in Hb and Hct was significantly greater for the YFC group per mg of iron consumed. ** p<0.01; ***p<0.001
Iron deficient rats were fed either YFC or ferrous sulfate for one week containing either 17mg/kg or 35mg/kg of iron. The amount of iron absorbed from the diets was monitored by subtracting iron consumed from iron in the feces. The animals had no other access to food than the prepared diets and were housed in open bottom cages so they could not consume the feces. The results of this study show that iron in YFC is absorbed better (almost 60%) at the low concentration compared to FeSO4 (40%) although the difference did not reach statistical significance. At the higher concentration iron absorbed from yeast and FeSO4 were similar at 50%. In the latter case FeSO4 iron absorption increased with increasing dose of iron but iron from the yeast decreased suggesting there is a regulatory limit to iron uptake from the yeast. (p>0.05. No significant difference between groups.)

3.2. Monkey Studies

To evaluate the potential of YFC providing a dietary source of iron in a second species, 18 ID monkeys were fed supplemental yeast daily for 1-2 months. At one month, Hgb values had increased significantly into the normal range (N = 18, p<.0002), with a further increment by the second month (N = 8, p<.0015). Improvement in the MCV was slower than for Hgb, but the modest increment evident at one month already attained statistical significance (N = 18, p<.016) and it then improved into the non-anemic normal range by end of the second month (N = 8, p<.0002). An additional 13 ID monkeys that served as controls across a similar 2-month period did not show significant changes in their hematology (Figure 7).

Figure 6. Iron deficient rats were fed either YFC or ferrous sulfate for one week containing either 17mg/kg or 35mg/kg of iron. The amount of iron absorbed from the diets was monitored by subtracting iron consumed from iron in the feces. The animals had no other access to food than the prepared diets and were housed in open bottom cages so they could not consume the feces. The results of this study show that iron in YFC is absorbed better (almost 60%) at the low concentration compared to FeSO4 (40%) although the difference did not reach statistical significance. At the higher concentration iron absorbed from yeast and FeSO4 were similar at 50%. In the latter case FeSO4 iron absorption increased with increasing dose of iron but iron from the yeast decreased suggesting there is a regulatory limit to iron uptake from the yeast. (p>0.05. No significant difference between groups.)

Figure 7. YFC improved iron status in primates. Young ID monkeys were given oral YFC supplements daily at 6 mg/kg. Hgb and MCV were measured after one or two months.
To demonstrate gut absorption and iron incorporation into RBC, YFC formulated with $^{57}$Fe was given to young ID monkeys for either 1 or 5 consecutive days (Figure 8). As compared to baseline levels prior to dosing, a single dose did not alter the $^{57}$Fe/$^{56}$Fe in RBC, either on Day 2 or 7. However, at one week there was significant enrichment of $^{57}$Fe in RBC after 5 daily doses, when provided at 6 mg Fe/kg body weight.

Figure 8. Iron from YFC is incorporated into RBCs in primates. YFC loaded with $^{57}$Fe was provided for 1 or 5 days to young ID monkeys. The $^{57}$Fe/$^{56}$Fe ratio in RBCs was determined by ICP-MS

4. Discussion

Three different mechanisms have been proposed for iron absorption from the gut. The most widely accepted is the absorption of elemental iron from digested food via the divalent metal transporter 1 (DMT-1) [24]. Other mechanisms must exist because DMT1 is not expressed in the gut in neonates until after nursing [25] and animals with DMT-1 mutations that limit iron uptake are still capable of absorbing sufficient iron to survive [26]. Heme is considered the most bioavailable form of iron in the diet [27], yet the mechanism of heme transport, if it is transported, has not been determined. A putative receptor for heme in the gut was originally proposed but later identified to primarily bind folate [28]. The mechanism of heme uptake in the gut remains an active area of study. Receptors for lactoferrin have been reported in the gut but the function of lactoferrin, if it is transported, has not been determined. A putative receptor for heme in the gut was originally proposed but later identified to primarily bind folate [28]. The mechanism of heme uptake in the gut remains an active area of study. Receptors for lactoferrin have been reported in the gut but the function of lactoferrin, if it is transported, has not been determined. A putative receptor for heme in the gut was originally proposed but later identified to primarily bind folate [28]. The mechanism of heme uptake in the gut remains an active area of study. Receptors for lactoferrin have been reported in the gut but the function of lactoferrin, if it is transported, has not been determined. A putative receptor for heme in the gut was originally proposed but later identified to primarily bind folate [28]. The mechanism of heme uptake in the gut remains an active area of study. Receptors for lactoferrin have been reported in the gut but the function of lactoferrin, if it is transported, has not been determined. A putative receptor for heme in the gut was originally proposed but later identified to primarily bind folate [28]. The mechanism of heme uptake in the gut remains an active area of study. Receptors for lactoferrin have been reported in the gut but the function of lactoferrin, if it is transported, has not been determined. A putative receptor for heme in the gut was originally proposed but later identified to primarily bind folate [28].

Our hypothesis that Fth1 is an iron delivery protein stems from our reports that Fth1 is taken up into the brain [37] and can replace transferrin as the iron source for oligodendrocyte development [38]. Our exploration into the possibility that Fth1 can be a dietary iron source was supported by the finding that it is enriched in breast milk [39]. Yeast transformed with H-ferritin were previously used in a model of rodent iron deficiency with similar results as in our study, but the H-ferritin gene was not stably incorporated into a chromosome [40]. Moreover, the iron content of the ferritin was not determined and was unlikely to be consistent in non-stably transformed yeast.

The conceptualization of ferritin as a nutritional iron source has received periodic interest in the past. Although different strategies for using ferritin in plants as an iron supplement have been attempted, it is important to note that soybean ferritin is very similar to the L-subunit of human ferritin, not the H-subunit we used in the current yeast formulation [19,35]. The studies using plant ferritin as a means of increasing dietary supplies of iron are no more efficient that iron salts [35,41]. Genetically modified rice over-expressing plant ferritin also showed iron absorption at rates no better than chemical compounds [41]. This approach is also problematic as much of the iron is associated with non-edible parts of the plant [20,42]. By incorporating H-ferritin into yeast, we are providing a stable and consistent form of food that can be introduced into any diet.

5. Conclusion

Ferrous sulfate is one of the oral iron supplements used most commonly to treat ID and anemia. Our studies show that YFC was well tolerated by both rats and monkeys and that consumption of a diet with YFC supplementation facilitated a progressive and safe recovery from anemia. Yeast are economical and a prevalent food source in many cultures. Our findings indicate that using nutritional yeast stably transformed to express H-ferritin could become a significant dietary iron source.

Acknowledgements

The monkey studies were funded in part by NIH R01 HD080201-01A1 (Coe, Lubach and Connor).

Statement of Competing Interests

Conflict of Interest: Connor and Keil are founding members of Sidero Bioscience and hold the patent on YFC. In addition to Connor and Keil, Flanagan, Unger and Patton are paid consultants of Sidero Bioscience.

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