

# HEMOGLOBIN-FORTIFIED BISCUITS: BIOAVAILABILITY AND ITS EFFECT ON IRON NUTRITURE IN SCHOOL CHILDREN<sup>1</sup>

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## SUMMARY

School-age children in Chile received 30 g of wheat-flour biscuits daily through a National School Lunch Program. To improve iron nutrition, these biscuits were fortified with 6% of a bovine hemoglobin concentrate. Hemoglobin iron bioavailability, measured with a double isotope technique, showed that heme-iron absorption in fortified biscuits was high (19.7%).

In a pilot field trial, a cohort of 215 school-children received fortified biscuits (30g) daily during two school periods, and their iron nutrition status was compared with that of children who received non-fortified biscuits (n=212). Acceptability of both types of biscuits was excellent. Initially, both groups had comparably good iron nutrition. The fortified children presented higher mean ferritin values at the end of the first and second school periods. Good iron stores (serum ferritin  $\geq 20\mu\text{g/l}$ ) were present in 92% and 79% of the fortified and control subjects, respectively ( $P < 0.004$ ).

The high-iron bioavailability, the good organoleptic characteristics and the biological effect on iron nutriture make this product an appealing alternative to combat iron deficiency.

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## INTRODUCTION

Iron deficiency continues to be prevalent throughout the world, affecting particularly infants, children and pregnant women (1). Food fortification with iron constitutes the most efficient and practical method for preventing iron deficiency in a community (2). Thus far, iron fortification has been carried out with inorganic salts or reduced iron particles (3); nevertheless, a major limitation of these fortificants lies in the decreased bioavailability induced by diets with a high content of inhibiting factors (4, 5). Several studies have determined that bioavailability of heme iron in the diet is higher than that of inorganic iron (6, 7). Additionally, the use of heme iron as a fortificant is appealing since bovine hemoglobin is largely wasted during the commercial slaughter operation. Industrial methods to collect and process the blood obtaining a bovine hemoglobin concentrate (BHC) had been developed in our laboratory (8), enabling us to initiate large-scale fortification trials.

In Chile, school children receive daily 30 g of biscuits (wheat flour) plus milk or a milk substitute beverage through a supplementary feeding program. This program is national in scope, reaches a significant proportion of the nutritionally vulnerable population and has enjoyed long standing support.

Previous national prevalence studies had shown that 7% of school children were anemic and 32% had transferrin saturation below 15% (9). Since biscuits were a daily component of the children's diet, it seemed to be the ideal vehicle to fortify with the bovine hemoglobin concentrate.

In this study, we measured the absorption of iron from BHC fortified biscuits and assessed its acceptability and biological effect on iron nutrition status in a school-children field study.

## MATERIAL AND METHODS

### *Characteristics of Heme Iron-Fortified Biscuits*

*Preparation of tagged and untagged bovine hemoglobin concentrates*—To obtain untagged BHC, bovine blood was collected in a bucket with sterile precautions at the time of slaughter with sodium citrate as anticoagulant to yield a final concentration of 2.5 g/l of blood. The collected blood was maintained at 5°C and separated within 3-6 hr into plasma and red blood cell fractions in a laboratory refrigerated centrifuge at 2000 x g for 30 min. The plasma was discarded and red cells were washed three times, with 0.9% NaCl. This preparation was freeze-dried in an Atlas LKB Autovac Gauge (3294B) at 30°C chamber temperature and 0.1 mm Hg chamber pressure (8).

To obtain intrinsically tagged hemoglobin, 4 mCi of <sup>55</sup>Fe in the form of ferric chloride (specific activity 0.15 mCi/mg) were injected intravenously into the jugular vein of a 7 day-old calf. Fifteen days later, the calf was slaughtered and radioactive ABHC (specific activ-

TABLE 1

## COMPOSITION OF BHC-FORTIFIED AND NON-FORTIFIED BISCUITS

	Fortified %	Non-fortified %
Wheat flour	55.3	61.0
Bovine hemoglobin concentrate (BHC)	5.7	0.0
Liquid sugar	28.6	28.6
Hydrogenated lard	9.5	9.5
Vanilla essence	0.15	0.15
Sodium bicarbonate	0.50	0.50
Monocalcium phosphate	0.15	0.15
Antioxidants	0.03	0.03

TABLE 2

PROXIMATE COMPOSITION AND IRON CONTENT OF  
BHC-FORTIFIED AND NON-FORTIFIED BISCUITS

	Fortified	Non-fortified
Moisture (%)	4.6	6.4
Protein (Nx6.25) (%)	13.5	8.4
Ether extract (%)	11.0	11.0
Ash (%)	1.1	1.0
Nitrogen-free extract (%)	69.8	73.3
Fe (mg/100g)	19.3	2.3

ity 4.3  $\mu\text{Ci}/\text{mg Fe}$ ) was prepared by freeze-drying the red cells as previously described (10).

*Biscuit composition* — Wheat flour biscuits were fortified with 6% of BHC (Table 1). Chemical analysis showed an eight-times higher iron content and a 1.6 greater protein content in the fortified biscuit. BHC fortification improved content of all essential amino acids, except isoleucine (Table 2).

*Apsorption Studies*

Fifteen well nourished children in apparent good health, ranging in age from 2 to 7 y (mean age=5.4 y) participated. Written, informed consent was obtained from both parents before the study. Two boys were anemic (hemoglobin <120 g/l). Iron status in the rest of the children was normal.

*Absorption measurements* — Iron absorption from BHC fortified biscuits and from a reference dose of ferrous ascorbate were measured. One kilogram of labelled biscuits was prepared using 60 g of a mixture of nonradioactive BHC with labelled BHC, to give an activity of 2.1  $\mu\text{Ci}$   $^{55}\text{Fe}$  per 30 g of biscuits. These tagged biscuits were hand-made and cooked in a domestic oven. On day 1, subjects received 30 g of the fortified biscuits containing 5.5 mg of elemental iron labelled with 2.1  $\mu\text{Ci}$   $^{55}\text{Fe}$ ; on day 2, they drank an aqueous solution of a standard containing 3 mg of iron as ferrous sulfite, in a 2:1 molar ratio of ascorbic acid to iron, labeled with 0.7  $\mu\text{Ci}$  of  $^{59}\text{FeSO}_4$  (New England Nuclear).

The preparations were consumed on consecutive mornings after an overnight fast, and no food and beverage other than water was permitted four hours thereafter. On day 15, venous blood samples were obtained to determine the hematologic characteristics of the subjects, and to measure the radioactivity incorporated into erythrocytes. Duplicate 10 ml blood samples and triplicate aliquots of the preparations ingested were processed for differential counting of  $^{55}\text{Fe}$  and  $^{59}\text{Fe}$  using the method of Eakins and Brown (11). The activity of radioisotopes in the processed samples was determined using a liquid scintillation spectrometer (Nuclear Chicago). The percentage absorption values were calculated from the *radioactivity present* in the circulating erythrocytes, assuming 70 ml/kg blood volume and 90% red cell utilization of radio iron (12). Serum iron and iron binding capacity were measured by the colorimetric method of Fischer and Price (13). Serum ferritin was quantitated using a radioimmunoassay (Travenol Laboratories, Inc, Cambridge, MA).

### *Field Trial*

A pilot field trial with the 6% BHC fortified biscuits was conducted in two schools in the city of Santiago, Chile. The two schools were selected on the basis of their location in a low income area of the city, having adequate coverage by the food supplementary program, and the willingness of parents and teachers to participate in the study. The latter had a duration of two school years (May-November 1980 and March-October 1981). Children in one school (Public School N<sup>o</sup> 181) received 30 g of BHC fortified biscuits (3 units) together with a glass of milk or milk substitute. In the control school (Public School N<sup>o</sup> 183), children continued receiving the standard non-fortified biscuits (30 g). Biscuits were given on school days only (approximately 180 days/year) and eaten under the supervision of a teacher to ensure consumption. Children were asked to leave the uneaten biscuits in the dining area. Once a week, a research nurse measured acceptance of the biscuit by direct observation of their consumption.

Iron nutrition status and anthropometry (weight and height) were measured in all six to nine year-old children and 10-12 year-old girls from each school (fortified and unfortified) (Table 3). They were evaluated at the beginning of the study (0 month), at the end of the first school period (7 months) and finally, at the end of the second

**TABLE 3**  
**AGE AND SEX AT THE BEGINING OF THE FOLLOW-UP**

Age (yrs)	Fortified group		Non-fortified group	
	Boys	Girls	Boys	Girls
6	13	18	21	20
7	26	26	18	19
8	27	23	30	21
9	19	16	16	22
10-12	—	47	—	45
<b>Total</b>	<b>85</b>	<b>130</b>	<b>85</b>	<b>127</b>

school period (15 months) of the study. Hemoglobin (Coulter model ZBI, Hialeah, FL), iron concentration and iron binding capacity (13), as well as serum ferritin (Travenol Laboratories Inc, Cambridge, MA), were measured.

The iron absorption studies and field trial protocols were in accordance with the standards of the Institute of Nutrition and Food Technology's Ethics Committee of Human Research. Furthermore, the radioactive test doses were approved by the Chilean Commission of Nuclear Energy.

## RESULTS

The mean iron absorption from BHC fortified biscuit was high, with a geometric mean of 19.7% (Table 4).

The two school-children groups, in the follow-up study, had a similar age and sex distribution (Table 3). Weight and height were also similar at the beginning and at the end of the follow-up. The percentage of subjects with a weight to height ratio below the 10th percentile of NCHS standards was below 2.5% in both schools.

The acceptability of the fortified biscuits was excellent. On the average, less than 2% of the served cookies were left uneaten throughout the two years of study, with no significant differences between the fortified and unfortified products (Figure 1).

There were no initial differences between the groups neither in hemoglobin concentration nor in serum ferritin; however, transferrin saturation was significantly higher in the non-fortified group (Table 5). Hemoglobin values were uniformly high in both groups, and no child had anemia (hemoglobin concentration at different ages according to Dallman and Siimes) (14). At the end of the first school period mean serum ferritin values were significantly higher ( $p < 0.05$ ) in the fortified group. At the end of the trial, there was a

**TABLE 4**  
**IRON ABSORPTION FROM BHC-FORTIFIED BISCUITS**  
**OBTAINED IN THE LABORATORY**

Subject No	Age years	Hb (g/l)	Fe/IBC (%)	SF* ( $\mu\text{g/l}$ )	Iron absorption (% of dose)		A/B
					Fortified biscuit (A)	Ferrous ascorbate (B)	
1	6	130	30.4	60	22.9	67.7	0.34
2	3	135	29.5	33	25.5	43.2	0.59
3	6	131	48.9	29	24.8	37.3	0.66
4	4	118	27.2	48	12.3	36.0	0.34
5	2	122	—	—	52.5	35.7	1.47
6	7	129	19.8	47	21.8	35.1	0.62
7	5	128	24.6	56	23.5	31.3	0.75
8	5	142	41.2	47	19.3	29.6	0.65
9	6	134	22.8	52	21.6	28.3	0.76
10	5	128	22.8	37	17.7	27.7	0.64
11	7	130	32.5	66	18.5	26.8	0.69
12	7	128	40.4	52	22.3	25.2	0.88
13	7	119	26.2	55	9.8	21.4	0.46
14	6	138	23.6	40	16.0	18.5	0.86
15	5	135	27.2	57	11.8	18.4	0.64
Mean	5	130	29.8	47	19.7*	30.4*	0.65
SD	2	6	8.1	38-59	13.4-29.0	22.0-42.0	—

\* Values are geometric means and range of 1 SD.

small, but statistically significant, higher hemoglobin concentration in the group consuming fortified biscuits.

When hemoglobin and serum ferritin were depicted using cumulative plots (Figures 2 and 3), no differences were evident at the initial evaluation. Nevertheless, there was a significantly lower percentage of subjects below 20  $\mu\text{g/l}$  of serum ferritin in the fortified group at 7 mo (13% v 25%,  $p < 0.04$ ) and at 15 mo (8% v 21%  $p < 0.004$ ). Serum ferritin values over 20  $\mu\text{g/l}$  were considered indicative of adequate iron stores.

## DISCUSSION

There are several reasons that favor the use of hemoglobin iron as an appealing alternative in the prevention of iron deficiency anemia: hemoglobin iron is better tolerated than non-heme iron;

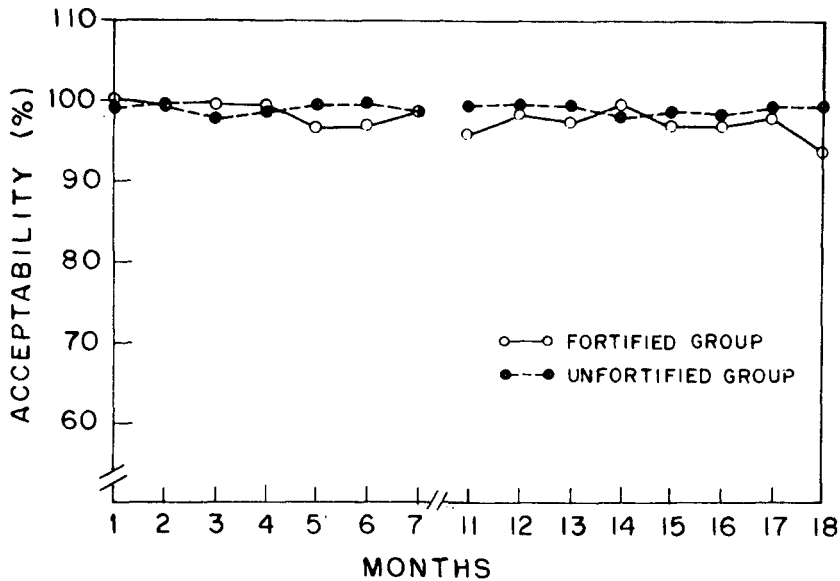


FIGURE 1

**Acceptability of fortified and control biscuits, expressed as the percentage of children consuming 30g of biscuits**

when added to test meals heme iron is better absorbed than non-heme iron and its absorption is not affected by food iron inhibitors (6, 7).

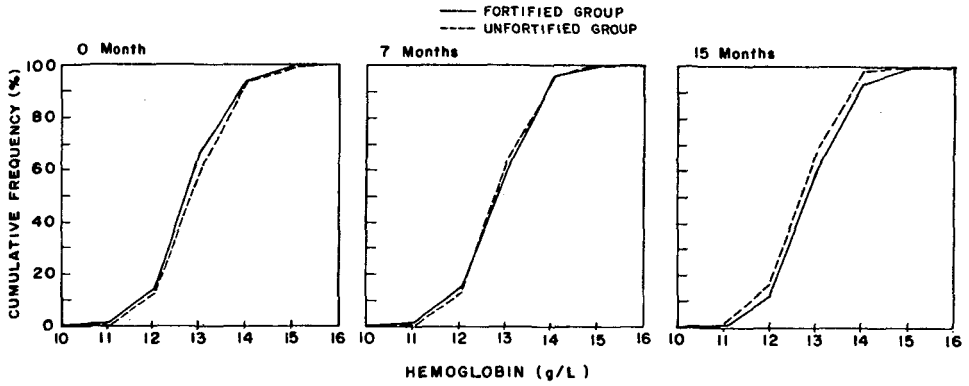
Because the diet of Chilean children consists mainly of cereal and vegetables, it was estimated that adding non-heme iron to their diet would not be significantly effective, especially when limited to the amounts that could be satisfactorily incorporated into a vehicle. Cereals (wheat flour and infant cereals) are fortified with electrolytic iron or iron salts. Recently, based on the relatively poor bioavailability of elemental Fe powder added to these vehicles, it has been suggested that this fortificant may be unsuitable for the prevention of iron deficiency in infancy (15). Iron salts, which present an adequate bioavailability (as FeSO), confer a very short shelf-life to the vehicle. An equivalent amount of hemoglobin iron, it was hypothesized, would be significantly more effective.

The use of hemoglobin as a food fortificant was suggested by Reizenstein in 1975 (16). Iron absorption from hemoglobin-fortified sausages, hamburgers, bread and liver paté was investigated in a very small group of subjects (17). The findings of these studies indicated that the use of hemoglobin for the fortification of meat products resulted in satisfactory iron absorption; however, when using bread as a vehicle, the bioavailability was poor. Nevertheless,

**TABLE 5**  
**HEMATOLOGIC VALUES FOR SCHOOL CHILDREN**  
**FROM 6 TO 12 YEARS OF AGE**

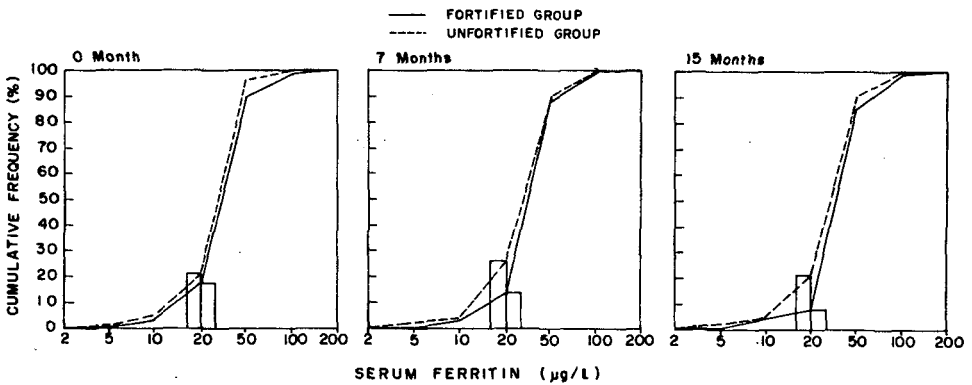
	Fortified group		Non-fortified group
<i>Hemoglobin (g/L)</i>			
<b>0 month</b>	<b>138.3 ± 7.5</b> <b>(212)*</b>	<b>NS</b>	<b>137.2 ± 7.6</b> <b>(210)</b>
<b>7 months</b>	<b>137.2 ± 7.4</b> <b>(207)</b>	<b>NS</b>	<b>137.5 ± 7.6</b> <b>(206)</b>
<b>15 months</b>	<b>136.5 ± 6.9</b> <b>(180)</b>	<b>&lt; 0.05</b>	<b>134.8 ± 8.2</b> <b>(169)</b>
<i>Fe/IBC (%)</i>			
<b>0 month</b>	<b>24.6 ± 8.0</b> <b>(206)</b>	<b>&lt; 0.01</b>	<b>26.8 ± 8.0</b> <b>(209)</b>
<b>7 months</b>	<b>27.6 ± 8.3</b> <b>(204)</b>	<b>NS</b>	<b>27.8 ± 8.7</b> <b>(206)</b>
<b>15 months</b>	<b>30.7 ± 8.8</b> <b>(176)</b>	<b>NS</b>	<b>31.4 ± 10.5</b> <b>(158)</b>
<i>Serum ferritin (µg/l) #</i>			
<b>0 month</b>	<b>27 (17-42)</b> <b>(120)</b>	<b>NS</b>	<b>27 (15-47)</b> <b>(106)</b>
<b>7 months</b>	<b>31 (20-47)</b> <b>(120)</b>	<b>&lt; 0.05</b>	<b>26 (15-47)</b> <b>(106)</b>
<b>15 months</b>	<b>31 (20-47)</b> <b>(120)</b>	<b>NS</b>	<b>28 (14-53)</b> <b>(106)</b>

\* Number of subjects. # Geometric mean and 1 SD range.



**FIGURE 2**

**Cumulative frequency distribution of individual values of hemoglobin at 0, 7 and 15 months of follow-up in school-children receiving fortified and non-fortified biscuits**



**FIGURE 3**

**Cumulative frequency distribution of individual values of serum ferritin at 0, 7 and 15 months of follow-up in school-children receiving fortified and non-fortified biscuits**

these studies have the serious methodological limitation given by the small number of subjects in the groups ( $n=3$  or  $4$ ). When hemoglobin iron was heated to the temperature necessary for the baking of bread, iron absorption was presumably reduced (17).

In 1977, Hertrampf, Amar and Steckel initiated investigations oriented to the possible use of hemoglobin in food fortification in Chile. Initially, the studies were focused on the fortification of milk (10). The bioavailability of hemoglobin iron was high (geometric mean 18.8%), in a group of iron-deficient infants. Hemoglobin added to liquid milk, however, produced rapid rancidity due to the oxidation of fat in the milk.

The existence of a National Program delivering biscuits to children in our country, in addition to their lower fat content, made biscuits a suitable vehicle for fortification with heme-iron.

Prior to the iron absorption studies, we established that fortification of biscuits with hemoglobin at a 6% concentration maintained good organoleptic characteristics for up to seven months when kept at ambient temperature, in oxygen and light-proof packaging (8).

The present study shows the high bioavailability of heme iron added to wheat-flour biscuits. When children consume 30 g of biscuits, the iron provided daily would be approximately 1 mg. This amount would supply the total iron requirements (18).

It should be noted that high bioavailability was obtained in spite of the baking process, and that the biscuits were not consumed with meat. Martínez-Torres and Layrisse (19), as well as Hallberg *et al.* (20) reported that heme-iron absorption is usually fairly poor in the absence of meat. The bioavailability of BHC is better than non-heme iron compounds generally utilized in the fortification of wheat flour and its processing products (21, 22). This better bioavailability is particularly important with respect to recent results obtained by Hallberg, Brune and Rossander (21), that show poor absorption of carbonyl iron compounds widely used in the fortification of cereals.

Our study shows the feasibility of the biscuit as a vehicle, since its consumption was constant and over a long period of time. Children given 30 g daily of BHC-fortified biscuits during two school periods increased their iron stores when compared to a control group. Other hematological changes were less evident, because the two groups of children had very good iron status at the beginning of the study. Based on these results, it would be predicted that the effect of BHC-fortified biscuits in a population with a poor iron status will be more evident.

The improvement of the total iron endowment obtained with the BHC-fortified biscuits in school-children will allow them to confront successfully the greater requirements imposed during adolescence.

In conclusion, we have established the high iron bioavailability of biscuits fortified with hemoglobin iron, its good acceptability, and its biological effect upon iron nutriture. These characteristics make it a promising product to be employed in the combat of iron deficiency.

## RESUMEN

**GALLETAS FORTIFICADAS CON HEMOGLOBINA:  
BIODISPONIBILIDAD Y EFECTO SOBRE LA NUTRICION  
DE HIERRO EN ESCOLARES**

En Chile, los escolares reciben diariamente 30 g de galletas de harina de trigo en un Programa de Desayunos Escolares. Estas galletas fueron fortificadas con 6% de hemoglobina de vacuno. La biodisponibilidad del hierro, determinada mediante una técnica dobleisotópica, mostró una elevada absorción del hierro hemínico en las galletas fortificadas (19.7%).

En un estudio piloto, a un grupo de 215 escolares se les administró diariamente la galleta fortificada (30 g) durante dos períodos escolares, comparándose su estrado nutricional férrico con 212 niños que recibieron galletas no fortificadas. La aceptabilidad de la galleta fortificada fue excelente. Al inicio ambos grupos presentaron una nutrición de hierro comparablemente buena. Al término del primer y segundo período escolar, el grupo fortificado acusó promedios de ferritina sérica más elevados. Los depósitos de hierro eran suficientes (ferritina sérica  $\geq 20 \mu\text{g/l}$ ) en el 92 y 79% de los sujetos fortificados, y controles, respectivamente ( $P < 0.004$ ).

La alta biodisponibilidad del hierro de esta galleta, sus óptimas condiciones organolépticas y su efecto sobre la nutrición de hierro, hacen de este producto una alternativa promisoría para la prevención de la deficiencia de hierro.

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