# FACTORS AFFECTING THE AVAILABILITY OF IRON IN WHEAT BRAN AND PEA BRAN

A Thesis

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How long the road is.

But,

for all the time the journey has already taken,

How you have needed every second of it

in order to learn

what the road passes by.

Dag Hammarskjold

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## ABSTRACT

The bioavailability of iron endogenous to high fiber muffins was evaluated in anemic rats using the hemoglobin regeneration technique. White wheat flour, pea bran, toasted pea bran (one hour at 177 C), α-cellulose, wheat bran, heated wheat bran (one hour at 100 C), toasted wheat bran (one hour at 177 C), enzymatically dephytinized wheat bran, and wheat bran that was boiled to prevent dephytinization were each incorporated into a white flour muffin batter. The muffins were baked, air-dried, ground, and incorporated into test diets to provide approximately 8% neutral detergent fiber. One white flour muffin diet served as a control treatment, while a second similar diet was fortified with $K_2HPO_4$ to provide a phosphorus content similar to that in the wheat bran diets. The α-cellulose and white flour diets were fortified with 18 ppm iron as ferrous sulfate. The bioavailability of iron was evaluated by comparing the hemoglobin-iron gain per intake of iron (hemoglobin efficiency) of the rats fed the test diets with the hemoglobin efficiency of rats fed a basal diet fortified with 18 ppm iron as ferrous sulfate. The ratio of the two efficiencies was referred to as the Relative Biological Value.

The Relative Biological Values assigned to the α-cellulose, white flour with added phosphorus, pea bran, and toasted pea bran diets (73, 81, 61, and 61, respectively) were significantly lower than those assigned to the 18 ppm diet (100). The Relative Biological Values associated with the wheat bran, heated wheat bran, and toasted wheat bran diets (91, 90, and 84, respectively) were not significantly different than the 18 ppm diet. There were no significant differences

between the dephytinized wheat bran, the boiled wheat bran, and the 18 ppm diets (101, 109, and 100, respectively).

There was no apparent relationship between iron bioavailability from the fiber sources investigated and the intake of phytate, phosphorus, lignin, or neutral detergent fiber. The intake of cellulose, acid detergent fiber, or crude fiber components may have influenced the iron availability of the α-cellulose, pea bran, and toasted pea bran diets.

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## ABBREVIATIONS

| | | |
|---|---|---|
| 0 ppm | = | Standard curve diet, unfortified |
| 9 ppm | = | Standard curve diet, fortified with 9 ppm iron as ferrous sulfate |
| 18 ppm | = | Standard curve diet, fortified with 18 ppm iron as ferrous sulfate |
| ADF | = | Acid detergent fiber |
| C | = | Degrees Centrigrade |
| CEL | = | α-cellulose |
| CF | = | Crude fiber |
| FeIn | = | Iron intake |
| Hb | = | Hemoglobin |
| HbEF | = | Hemoglobin efficiency |
| HbFe | = | Hemoglobin-iron |
| HbFeG | = | Gain in whole body hemoglobin-iron |
| HWB | = | Heated wheat bran |
| NDF | = | Neutral detergent fiber |
| PB | = | Pea bran |
| +PWB | = | Phytate-maintained wheat bran |
| -PWB | = | Phytate-reduced wheat bran |
| RBV | = | Relative biological value |
| SE | = | Standard Error of the Mean |
| TPB | = | Toasted pea bran |
| TWB | = | Toasted wheat bran |
| WB | = | Wheat bran |
| WF | = | White wheat flour |
| $WF+PO_4$ | = | White wheat flour + $K_2HPO_4$ |

# 1. INTRODUCTION

Iron is essential for human health. Iron-containing compounds including hemoglobin, myoglobin, hemeprotein enzymes, and iron-flavoproteins are required for physiological processes including oxygen transportation and energy utilization.

Iron status is ultimately dependent upon the balance of iron losses, increased iron requirements, and iron absorption. Failure to meet the physiological needs for iron results in the progressive decrease of body iron, culminating in iron deficiency anemia. The widespread prevalence of iron deficiency anemia indicates that iron balance is not always attained. Negative iron balance in developed countries has been attributed primarily to inadequate iron intake, while poor iron status in developing countries appears to be related to the low availability of iron in the diet.

Although most diets contain several times the physiological requirement for iron, only a fraction of the iron consumed is actually absorbed. The per cent iron absorption, which averages ten per cent in a mixed diet, is influenced by the ionic nature and chemical form of the iron, as well as other constituents in the diet. The nature and degree of food processing may also affect iron availability.

Wheat bran represents a rich source of dietary iron. However, the availability of wheat bran iron to humans is controversial. A greater understanding of the absorption of wheat bran iron is critical as whole wheat breads can contribute up to 90% of the dietary energy consumed by certain Middle Eastern and East Indian populations. While cereal products make a much smaller contribution to the intake of most populations in developed countries, the consumption of

fiber-rich wheat bran products in these countries is on the rise.

Field pea bran, which is a popular new source of fiber in Canadian markets as well as abroad, has yet to be investigated for its effect on iron absorption. Pea bran is primarily incorporated into bread products, a group of items from which many people receive the majority of their minerals. In the event that pea bran inhibits iron absorption, the presence of pea bran in the diet could be expected to upset an already precarious iron balance.

The purpose of this study was to estimate the availability of the iron endogenous to wheat bran and pea bran using anemic rats as a model for human iron absorption. The fibers were baked into muffins to simulate the fiber sources as they are typically found in the human diet. The individual effects of bran components including phytate, phosphorus, neutral detergent fiber, acid detergent fiber, crude fiber, cellulose, hemicellulose, and lignin were investigated.

## 2. LITERATURE REVIEW

### 2.1 Iron in the Body

The iron content of the human body varies with the subject's weight, sex, hemoglobin concentration, and size of iron stores. The average adult male maintains a level of 50 mg iron/kg body weight, while the adult female contains 35 mg iron/kg (Beutler, 1980).

Seventy per cent of the iron in the body is considered to be functional iron, which includes metabolic and enzymatic compounds such as hemoglobin, myoglobin, heme-enzymes, and several other proteins that function in the transport, storage, and utilization of oxygen. Hemoglobin contains 85% of the functional iron, while five per cent is found in myoglobin, and ten per cent in the heme-enzymes. Four mg of iron are bound to transferrin in the plasma (Beutler, 1980). The remaining 25 to 30% of body iron is considered non-essential, and is located in the spleen, liver, and bone marrow in the form of the storage compounds, ferritin and hemosiderin (INACG, 1979).

Iron is essential for human health. Heath et al. (1932) demonstrated that inorganic iron can be incorporated into hemoglobin. The ability of iron to be reversibly oxygenated allows hemoglobin to transport oxygen from the lungs to the body tissues. The heme-protein enzymes and the iron-flavoprotein enzymes are also intimately involved with oxygen utilization (Mahler and Elowe, 1953; Richert and Westerfeld, 1954). Ferritin and hemosiderin function as an iron reserve, releasing storage iron into the bloodstream during periods when iron intake is inadequate to meet metabolic needs (Bothwell et al., 1979).

## 2.2 Iron Homeostasis

The human body constantly runs the risk of developing a negative iron balance. To maintain iron homeostasis, the body must balance iron absorption with iron loss and increased physiological requirements (Beutler, 1980).

### 2.2.1 Iron Absorption

The mechanism of iron absorption has been the subject of several reviews (Forth and Rummel, 1973; Narasinga Rao, 1981; Charlton and Bothwell, 1983).

Three distinct phases in iron absorption have been elucidated (Narasinga Rao, 1981). During the first intraluminal phase, food is digested and iron is released in a soluble form. The acidic gastric juice secreted by the stomach plays a critical role in the ionization of iron as well as the reduction of ferric ions to the more soluble ferrous ions (Bezwoda et al., 1978). During the mucosal phase, iron is taken up by the mucosal cell. Although iron absorption can be demonstrated to occur in the stomach, the ileum, and the colon, the majority of absorption occurs in the duodenum and the upper jejunum (Wheby, 1970). The final, or corporeal, phase involves the uptake of iron by transferrin on the serosal side of the mucosal cell, and the subsequent transport to the liver and hemopoietic tissues.

The mechanism responsible for controlling iron absorption may operate at one or more of several loci within the luminal epithelium of the intestinal mucosa, including the brush border membrane, the intracellular sites of iron transfer, or the level of efflux across the basolateral membranes (Cox and O'Donnell, 1982). Bogunjoko et al. (1983) suggested that the rate of stomach emptying may also affect

iron absorption rates. The slower stomach emptying that was observed in iron-deficient rats compared to iron-replete rats may allow increased contact time between the iron and the mucosa, thereby increasing iron absorption.

2.2.2 Iron Loss

Once absorbed, the store of iron in the body is meticulously guarded; over 90% of the total body iron is recycled (Beutler, 1980). Basal losses in the form of desquamated cells, urine, sweat, nails, and feces amount to only 0.7 to 0.8 mg iron/day in females, and 0.9 to 1.0 mg iron/day in males (Green et al., 1968). However, additional avenues for iron loss do exist. Menstruation, parasitic infestation, hemorrhage, gastrointestinal bleeding, and blood donations all result in the loss of body iron (Bothwell et al., 1977). Blood loss from any body site constitutes a loss of 0.5 mg iron/mL blood (Beutler, 1980).

2.2.3 Increased Iron Requirements

Periods of rapid growth, such as infancy, adolescence, and pregnancy, are associated with large increases in blood volume. To allow for the expansion of hemoglobin stores, iron requirements during these periods are dramatically increased (Beutler, 1980).

Although the human infant is born with a substantial store of iron, the rapid rate of growth during the first year of life draws heavily upon the iron reserves. The infant requires 0.5 to 1.5 mg of absorbable iron/day, a need that is several times higher per unit body weight than the iron needs of the human adult (Committee on Iron Deficiency, 1968). Unless the dietary supply of iron is adequate, the iron stores of the infant may be depleted as early as four months of age. The highest prevalence of iron deficiency anemia occurs between

six months and two years of age (INACG, 1979).

The acceleration of growth associated with adolescence imposes increased iron requirements during the teenage years. The onset of menses in females and the increase in blood hemoglobin concentration in males further increase iron requirements (INACG, 1979). A total of one to 2.7 mg iron/day should be absorbed to provide for teenage growth and sexual development (Finch et al., 1968).

Pregnancy and lactation also increase the need for iron. Although the pregnant woman no longer loses iron through menstruation, the cost of pregnancy in terms of iron needs is high. The fetus obtains iron transplacentally at the expense of maternal stores (MacPhail et al., 1980). The growth of umbilical and placental tissue, the expansion of maternal red cell mass, and the loss of maternal blood at delivery also add to the expense of pregnancy. As a result, iron requirements are increased over and above the level needed to maintain balance prior to pregnancy. During the second and third trimesters, the mother may require a total of five mg absorbable iron/day to meet her needs and maintain body stores. Lactating mothers require an additional 0.1 to 0.3 mg absorbable iron/day to replace the iron being secreted in the milk (INACG, 1981).

### 2.2.4 Iron Balance

Iron status is ultimately determined by the balance of iron uptake, iron loss, and increased physiological requirements (WHO, 1975). As the body's capacity to excrete iron is extremely limited, iron balance is believed to be regulated primarily through the control of iron absorption (McCance and Widdowson, 1937; Beutler, 1980). The iron uptake required to maintain hematological parameters varies with

age, sex, and physiological status (Bothwell et al., 1979).

The adult male can easily replenish daily basal losses by eating a balanced and varied western type diet (RDA, 1974). Absorption of only six per cent of the dietary iron is required to maintain iron stores in males (Bothwell et al., 1979). In contrast, certain segments of the population have difficulty maintaining a positive iron balance. Unfortified infant formulas often contain iron levels that are insufficient to meet the high physiological demands of infancy (INACG, 1979). Breastfeeding offers some protection against iron depletion, for the small quantity of iron that is present in breastmilk is highly available to the infant. However, iron status may be compromised by the use of unfortified milk formulas or the early transition from breastmilk to unfortified weaning foods. Dallman et al. (1980) recommended the provision of either iron-fortified cereals or a total supplement of 200 mg iron during the second half of infancy.

Due to menses, the female of child-bearing age loses the equivalent of 0.5 to 0.8 mg iron/day over and above basal losses (Underwood, 1977). In order to maintain iron balance, the average American woman absorbs approximately 15% of the iron in the diet (Bothwell et al., 1979). The inability of women to fulfill their iron requirements during the second and third trimesters of pregnancy is well documented (INACG, 1981). The proportion of pregnant women with a hemoglobin concentration of less than 11 g/dL ranged from 22% in Poland to 56% in Vellore, India and 84% in Burma (Baker and DeMaeyer, 1979). Iron status is difficult to maintain during pregnancy without the use of iron supplements. The National Research Council recommended

the supplementation of 30 to 60 mg of elemental iron/day during the final two trimesters of pregnancy (RDA, 1980).

## 2.3 Iron Deficiency

Failure to meet the physiological needs for iron results in the progressive decrease of body iron. Initially, iron stores become depleted, and the concentration of serum ferritin drops from 70 ug/L to less than 12 ug/L. The second phase, iron deficient erythropoeisis, is marked by a decrease in metabolic iron. The per cent transferrin saturation drops from 30% to less than 15%, and the free erythrocyte protoporphyrin rises to above 70 ug/mL. With continued iron depletion, the incorporation of iron into hemoglobin is compromised to the extent that the hemoglobin concentration drops below the normal physiological range (Cook and Finch, 1979). Microcytic, hypochromic anemia is the result of severe iron deficiency (Beutler, 1980).

### 2.3.1 Prevalence

Iron deficiency is the most commonly recognized form of nutritional deficiency in the world (WHO, 1975; INACG, 1981). The prevalence of iron deficiency anemia has been documented in developing countries as well as affluent societies (INACG, 1979; Nutrition Canada, 1975).. Garby (1973) estimated that the prevalence of iron deficiency was as high as 30 to 50% amongst the populations of developing nations, while the second National Health and Nutrition Examination Survey (NHANES II, 1976-1980) diagnosed 5.7% 5.9% and 5.8% of American infants, teenage girls, and young women, respectively, as having iron deficiency anemia (Dallman et al., 1984). Shah and Belonje (1976) measured the liver iron status of Canadians and found iron stores of less than 50 ug/g liver in 20% of the females and five

per cent of the males tested. Although Nutrition Canada (1975) documented few cases of frank anemia, the number of Canadians surveyed who were considered to be at moderate to high risk of iron depletion ranged from 2.7% amongst the 10-19 year old females to 4.5% of the 0-4 year olds, 6.8% of the pregnant women, and 19% of the males 65 years or older.

### 2.3.2 Deleterious Effects

Iron deficiency causes few deaths. However, this condition is thought to contribute to the weakness, ill health, and substandard performance of millions of people (Beutler, 1980).

The decrease in hemoglobin concentration induced by iron deficiency compromises the oxygen-carrying capacity of the blood, reducing the delivery of oxygen to the body tissues. As a result, severe iron deficiency anemia has been shown to impair the maximal work capacity in humans and rats (Viteri and Torún, 1974). Even small decreases in hemoglobin were capable of decreasing performance during exercise (Gardner et al., 1977). Severe iron deficiency in pregnant women also increases the risk of morbidity and mortality for both the mother and child. Mild anemia may increase the risk of premature delivery, low birthweight, placental hypertrophy, and reduced estriol secretion (INACG, 1977).

Although anemia is the most commonly observed symptom of iron deficiency, nonhematological effects are becoming increasingly evident. Muscle dysfunction observed in iron-deficient rats was attributed to decreased concentrations of the iron-containing enzyme, muscle α-glycerophosphate oxidase (Finch et al., 1976). Canale and Lanzkowsky (1970) observed hypoplasia and hypertrophy of the liver,

hypertrophy of the kidney, and hypoplasia and hypotrophy of the spleen in iron-deficient rats, indicating abnormal cell growth and morphology.

Although the effect of iron deficiency on resistance to infection remains uncertain, Chandra et al. (1977) concluded that the human system is very sensitive to hemoglobin concentration, and responds adversely to even slight deficiencies. Alterations in the immunological systems associated with iron deficiency include impairment of lymphocyte transformation, decreased production of migration inhibition factor, and cutaneous delayed hypersensitivity (Baker and DeMaeyer, 1979). Iron deficient subjects also displayed an impairment of the humoral-antibody response which decreases host resistence to bacterial infection. Chandra (1973) observed that children suffering from iron deficiency displayed reduced intracellular bacteria killing of Staphylococcus aureus, and attributed the impairment to a reduction in leucocyte myeloperoxidase.

Well-known and accepted clinical symptoms of iron deficiency include angular stomatitis, glossitis, dysphagia, hypochlorhydria, koilonychia, pica, and papilladema. Other symptoms, including listlessness, fatigue, irritability, and anorexia are commonly associated with iron deficiency, but the relationship remains unproven (Oppenheimer and Hendrickse, 1983).

### 2.3.3 Etiology

The factors involved in the development of iron deficiency are often complex and interrelated. Iron deficiency is most likely to occur during periods when iron requirements are greatest, such as infancy, adolescence, the child-bearing years of women, and pregnancy

(WHO, 1975). Factors including economic status, religious beliefs, geographical location, eating practices, and food choices may restrict iron absorption, thereby increasing the risk of iron inadequacy (Bothwell et al., 1979).

In developing countries, where the intake of iron often exceeds the dietary requirements, iron deficiency is due primarily to poor iron availability (Hallberg et al., 1983). In developed countries, poor iron status is more often the result of low dietary intakes. Canadian adolescents and adult females had median iron intakes in the marginal range, while 23.3% of 0-4 year olds and 29.7% of pregnant women reported inadequate iron intakes (Nutrition Canada, 1975). In many parts of the world, the increased loss of blood due to parasitic infestations may also contribute to negative iron balance (Bothwell et al., 1979).

## 2.4 Iron Bioavailability

Nutrient bioavailability may be defined as the proportion of a nutrient that is absorbed, transported to its site of action, and incorporated into physiologically active compounds. This concept is critical in the study of minerals, as the quantity available for metabolic purposes is often only a fraction of the minerals consumed in the diet (Mertz, 1983; O'Dell, 1983; Hallberg, 1981).

The bioavailability of iron in most diets is low, due to the progressive reduction in the quantity of iron that is available at each stage of absorption. Of the 15 mg of iron found in a western type diet, less than half is released in the soluble form. Three mg are taken up by the mucosal cells, and only 0.9 mg is transferred into the plasma (Bothwell et al., 1979). Although the absorption of iron

from individual meals may vary markedly, only five to ten percent of the iron in a mixed diet is available to the body (Beulter, 1980).

## 2.5 Measurement of Iron Availability

Iron bioavailability has been investigated using a wide variety of methods and species. Several authors have reviewed the techniques involved (Narasinga Rao, 1981; Hallberg, 1981; Morck and Cook, 1981; Turnlund, 1982).

### 2.5.1 In Vitro Methods

Although *in vitro* methods have been used for over fifty years, the validity of early *in vitro* techniques has been questioned (Narasinga Rao and Prabhavathi, 1978). The measurement of ionizable iron in food samples was inappropriate as this method failed to account for the conditions in the stomach and duodenum that influence iron availability. The use of only a simulated gastric digestion may also be invalid, as the majority of iron is absorbed in the duodenum, where the solubility of iron decreases markedly (Wheby, 1970). More recently, Narasinga Rao and Prabhavathi (1978) developed a technique that simulated the conditions in both the stomach and duodenum. The ionizable iron that remained following a sequence of enzyme treatment at pH 1.35 and an increase in pH to 7.5 was highly correlated with *in vivo* iron absorption in humans.

### 2.5.2 In Vivo Methods

#### 2.5.2.1 Chemical Balance

Iron absorption in the chemical balance method is defined as the difference between oral iron intake and fecal iron excretion. The major limitation is that such small quantities of iron are absorbed relative to the total iron intake, resulting in as little as five to

ten per cent difference between the ingested and excreted iron. In addition, the unabsorbed iron of dietary iron can not be distinguished from the iron of endogenous origin that is also excreted in the fecal material. The time-consuming, imprecise chemical balance method is now primarily of historical interest (Bothwell et al., 1979; Hallberg, 1981).

2.5.2.2 Isotopic Methods

Radioisotopes have been used to measure iron absorption in humans for over forty years (Hahn et al., 1945). Techniques which exploit the radioactive nature of radioiron to measure iron absorption include radioiron balance, the determination of red cell radioiron, and whole body counting (Bothwell et al., 1979). Although the measurement of whole body retention of radioiron is considered to be highly reliable, sensitive, and quantitative, the studies are expensive, complicated, and time-consuming (Narasinga Rao and Prabhavathi, 1978). In addition, studies are limited in the levels of radioisotopes used as well as the populations that can be exposed to radioactivity (Turnlund, 1982).

More recently, stable isotopes have been applied to the measurement of iron availability. Turnlund (1982) suggested that stable isotopes may provide an attractive alternative to methods involving radioisotopes.

2.5.2.3 Hemoglobin Regeneration

The iron-replete human incorporates 80% of absorbed iron into hemoglobin (Hosain et al., 1967). Hallmans et al. (1983) detected ten per cent of the absorbed $^{59}Fe$ in the liver tissue of normal rats, indicating that less than 90% of absorbed iron was available for hemoglobin synthesis. In contrast, Miller (1982) reported that

severely anemic rats fed diets with low to moderate concentrations of iron incorporated essentially all of the absorbed iron into hemoglobin, until blood iron levels of 0.4 mg/mL or the equivalent hemoglobin concentration of 12 g/dL was achieved.

The official AOAC method (1980) for determining iron availability (43.190) measures the ability of a test source of iron to regenerate the hemoglobin concentration of anemic rats. Male weanling rats are fed a low iron diet until the mean hemoglobin concentration drops to six g/dL. Four groups of rats are fed the basal diet to which 0, 6, 12, or 24 ppm were added as ferrous sulfate (standard reference diets). Each test source of iron is incorporated into the basal diet at three different levels, designated to provide hemoglobin responses in the same range as those produced by the standard reference diets. The repletion diets are fed for two weeks, after which the blood hemoglobin responses associated with the test diets are compared to those associated with the standard reference diets. The results are interpreted using the analysis for parallel lines-type assays. The availability of the test source iron, expressed as a percentage of the standard reference hemoglobin response, is termed the Relative Biological Value (RBV).

Use of the final hemoglobin concentration as the sole indication of iron availability may be inappropriate if parameters including the initial rat weight, weight gain, feed intake, and dietary iron level vary significantly between the test groups (Mahoney et al., 1974; Miller, 1977). The rat incorporates absorbed iron into hemoglobin that serves to maintain or increase the hemoglobin concentration of the initial blood volume. However, the total blood volume in the rat

varies directly with body weight (Cartland and Koch, 1928; Whittacker et al., 1984). As a result, the growing rat must also incorporate iron into hemoglobin to allow for the expansion of blood volume. The small rat, with a correspondingly low blood volume, will demonstrate a greater hemoglobin response to a given quantity of iron than a larger rat. Similarly, the rat that gains little weight during the regeneration period will exhibit a greater hemoglobin response to the absorption of a given quantity of iron compared to the animal that gains several grams.

Upon comparing the results obtained using the final hemoglobin concentration, the change in hemoglobin concentration, and the net hemoglobin-iron gain, Miller (1977) concluded that the latter measurement provided the best measure of response. Similarly, Miller (1982) reported that hemoglobin-iron gain was more highly correlated to iron intake than the blood hemoglobin concentration. Hemoglobin-iron gain was highly correlated to carcass iron gain up to a blood hemoglobin concentration of 12 g/dL.

Differences in diet intake due to palatability problems or nutritional inadequacies can be expected to alter the quantity of iron that is accessible to each of the test groups. Differences in the concentration of iron in each diet may also affect the quantity of iron that is consumed within each test group. Therefore, the measurement of iron bioavailability should account for differences in total iron intake during the regeneration period (Miller, 1977).

A wide variety of hemoglobin repletion techniques have been reported in the literature (Amine and Hegsted, 1974; Miller, 1977; Ranhotra et al., 1981). Differences in one or more experimental