

Effect of Iron-Food Intake on Anaemia Indices; Haemoglobin, Iron and Ferritin among Childbearing Egyptian Females

¹M.K. Abdel-Rahman, ²A. Aboul Anein and ²A.M. Hussien

¹Department of Nutrition and Food Science, Faculty of Home Economics, Helwan University, Egypt

²Department of Biochemistry, Faculty of Agriculture, Cairo University, Egypt

Abstract: This study was aimed at evaluating the effects of different plant sources and animal liver on iron intake on childbearing Egyptian females who suffer from nutritional anemia. The study included 25 childbearing Egyptian females between the ages of 25–40 years. The current study used anemia cut-off values below the normal levels of 12 g/dl, 41 µg/dl, and 20 µg/l for hemoglobin, iron, and ferritin, respectively. All females provided full consent for participation in the study. The participants were classified into 5 groups; each group consisted of 5 females ingesting 100 grams daily of either a plant or animal iron source for 7 weeks. The plant sources were Egyptian folk foods that are believed to improve anemia, such as aubergine (*Solanum melongena*), black dates (*Phoenix dactylifera*), blackstrap molasses, watercress (*Nasturtium officinale*), and buffalo liver. Before and after every week, hemoglobin, iron, and ferritin levels were determined. In summary, the results showed that aubergine, black dates, and buffalo liver improved hemoglobin, iron, and ferritin levels compared with watercress and molasses. Many nutritional factors may improve anemia and, therefore, further study is needed. The researchers recognize the importance of defining the relationship between iron intake and anemia among childbearing Egyptian females. In this study, the results indicated that both plant and liver sources of iron can enhance levels of serum anemia indices, which increase the ratio of hemoglobin, iron, and ferritin in serum.

Key words: Anaemia • ferritin • iron • haemoglobin • childbearing females • blackstrap molasses • black dates • aubergine • watercress • buffalo liver

INTRODUCTION

Iron, one of the most abundant metals on Earth, is essential to most life forms and to normal human physiology. Iron is an integral part of many proteins and enzymes that maintain good health. In humans, iron is an essential component of proteins involved in oxygen transport [1, 2] It is also essential for the regulation of cell growth and differentiation [3, 4]. A deficiency of iron limits oxygen delivery to cells, resulting in fatigue, poor work performance and decreased immunity [2, 5, 6]. On the other hand, excess amounts of iron can result in toxicity and even death [7]. Developing countries are the highest prevalence of iron deficiency. According to the data collected by WHO indicated that a total of 149 million people are iron-deficient or anaemic [8], 83 million of them anaemic women [9]. Iron deficiency anemia remains the most common single nutrient disorder worldwide. It is associated with several deleterious consequences,

including anemia, increased susceptibility to infection and impaired cognitive development and learning ability in children. Persistent iron deficiency during childbearing may have long-term consequences into anemic babies. The definition of optimal iron intake and status for human health is an issue of considerable controversy and strategic importance.

Plant foods contain almost all of the mineral and organic nutrients which are established as essential for human nutrition in addition to a number of unique organic phytochemicals that have been linked to the promotion of good health [10] In Egypt, the major sources of iron are plants as they consumed from, legumes, leafy green vegetable and fruits, molasses blackstrap and some animal foods. Animal sources are good sources of iron but for a financial reason the plant sources are the starkest source of iron in Egypt.

Some plant foods are better sources of iron than others, being higher in iron concentration and/or

containing food components that enhance iron bioavailability. Interestingly, this diversity in composition occurs not only between plant species, but also within genotypes of a single species.

One of the key causes of iron deficiency is poor bioavailability of iron in the diet, particularly in diets based mostly on rice, corn and wheat, with little dietary consumption of meats, fruits and vegetables.

Iron sources: Heme iron is found only in meat, fish and poultry and is absorbed much more easily than Non-heme iron, which is found primarily in plant sources. For example, one tablespoon of molasses blackstrap contains 2.3 mg iron and 3.0 servings of beef liver contain 7.5 mg of iron [11]. Increasing the amount of bioavailable micronutrients such as iron in plant foods for human consumption is a challenge especially in developing countries where plant foods comprise a significant portion of the diet.

Iron deficiency marker: Iron deficiency develops gradually and usually begins with a negative iron balance, when iron intake does not meet the daily need for dietary iron. This negative balance initially depletes the storage form of iron while the blood haemoglobin level, a marker of iron status, remains normal. Iron deficiency anaemia is an advanced stage of iron depletion. It occurs when storage sites of iron are deficient and blood levels of iron cannot meet daily needs. Blood haemoglobin levels are below normal with iron deficiency anaemia [2].

Subjects and methods: Childbearing Egyptian female volunteers were diagnosed as anaemic with cut off point of anaemia indices were less than 12 g/dl, 41 µg/dl and 20 µg/l for haemoglobin, iron and ferritin respectively. Control group consisted of 6 females with normal level of haemoglobin, iron and ferritin 15 g/dl, 80 µg/dl and 100 µg/l respectively. After a full consent to participate in this study, physical examination, anthropometric measurements, socioeconomic and dietary intake data were collected by using a questionnaire. Serum haemoglobin (Hb), ferritin and iron were assessed using standard laboratory kit methods (Randox Laboratories Ltd UK).

Preparation of foods: 100 grams of the following food items were ingested between meals; aubergine, black dates, molasses blackstrap, watercress and buffalo liver. Foods were prepared and eaten according to the Egyptian food habits. Aubergine and buffalo liver were served fried and grilled respectively.

Serum preparation: Peripheral venous blood obtained from fasting subjects was placed into 10 ml glass tubes. Serum was isolated by tube standing for 30 minutes and centrifuged at 950 xg for 10 min at 4°C and transferred into 1ml aliquots; stored at -70°C until required.

Chemicals: Unless otherwise stated, all chemicals were purchased from Sigma. All chemicals were 'Analar' grade or the highest grade available.

Iron colorimetric method: Iron was determined by using colorimetric method; *in vitro* diagnostic kit according to Dreux [12]. A 500µl of serum (free of haemolysis) was used.

Ferritin: Serum ferritin was determined by using colorimetric method *in vitro* diagnostic kit according to Bernard and Lauwerys [13]. A 100µl of serum (free of haemolysis) was used.

Haemoglobin colorimetric method: Haemoglobin was determined by using colorimetric method; *in vitro* diagnostic kit according to Van Kampen and Zijlstra [14] and recommendations of International Committee for Standardization in Haematology [15]. A 20µl of blood sample (free of haemolysis) was used.

RESULTS

In Table 1 there was an improvement in haemoglobin iron and ferritin compared to the baseline in 0 week. The improvement of anaemia parameters were calculated as a percentage in week 7 compared to week 0 was as 116, 174 and 232%, for haemoglobin, iron and ferritin respectively. Therefore, level of ferritin achieved the highest percentage.

In Table 2 there was an improvement in haemoglobin, iron and ferritin compared to the baseline in 0 week. The improvement of anaemia parameters were calculated as a percentage in week 7 compared to week 0 as 115, 149 and 194%, for haemoglobin, iron and ferritin respectively. Therefore, the level of ferritin achieved the highest percentage of an improvement than other indices.

In Table 3 there was an improvement in haemoglobin, iron and ferritin compared to the baseline in 0 week. The improvement of anaemia parameters were calculated as a percentage in week 7 compared to week 0 was as 110, 130 and 209% for haemoglobin, iron and ferritin respectively. Therefore level of ferritin achieved the highest percentage of improvement than other indices.

Table 1: Effect of ingesting buffalo liver (100g) on haemoglobin, iron and ferritin levels among anaemic childbearing Egyptian females

week	Haemoglobin (g/l)	Iron (μ g/dl)	Ferritin (μ g/l)
0	9.47 \pm 0.22	27.33 \pm 5.49	15.33 \pm 1.20
1	10.57 \pm 0.55	36.33 \pm 8.99	17.67 \pm 2.40
2	11.10 \pm 0.75	50.33 \pm 15.03	33.33 \pm 10.48
3	10.00 \pm 1.32	40.00 \pm 15.04	34.33 \pm 14.50
4	10.57 \pm 0.74	40.00 \pm 13.00	32.67 \pm 9.35
5	10.60 \pm 0.80	41.67 \pm 12.81	34.33 \pm 9.28
6	10.20 \pm 0.35	33.00 \pm 6.03	25.00 \pm 4.00
7	11.03 \pm 0.48	47.67 \pm 9.60	35.67 \pm 9.49

All values are the mean of 3 measurements

Table 2: Effect of ingesting Molasses blackstrap (100g) on levels of haemoglobin, iron and ferritin among anaemic childbearing Egyptian females

week	Haemoglobin (g/dl)	Iron (μ g/dl)	Ferritin (μ g/l)
0	9.90 \pm 0.46	35.67 \pm 2.40	15.67 \pm 0.88
1	11.20 \pm 0.42	43.67 \pm 3.28	22.67 \pm 4.67
2	12.00 \pm 0.50	58.00 \pm 5.86	27.67 \pm 5.78
3	11.70 \pm 0.23	57.67 \pm 5.24	26.33 \pm 2.60
4	10.70 \pm 0.32	43.33 \pm 3.84	25.00 \pm 2.65
5	11.67 \pm 0.33	52.67 \pm 3.18	29.33 \pm 2.03
6	11.50 \pm 0.10	52.00 \pm 2.31	30.00 \pm 0.58
7	11.40 \pm 0.10	53.00 \pm 2.89	30.33 \pm 1.45

All values are the mean of 3 measurements

Table 3: Effect of ingesting watercress (100g), *Nasturtium officinale* (Cruciferae) on haemoglobin, iron and ferritin levels among anaemic childbearing Egyptian females

week	Haemoglobin (g/dl)	Iron (μ g/dl)	Ferritin(μ g/l)
0	10.2 \pm 0.30	36 \pm 0.88	17 \pm 0.58
1	10.97 \pm 0.30	41 \pm 0.67	19 \pm 0.33
2	10.97 \pm 0.63	52 \pm 6.36	29 \pm 5.78
3	10.80 \pm 0.45	50 \pm 5.33	28 \pm 4.81
4	10.23 \pm 0.62	44 \pm 5.36	26 \pm 4.04
5	11.03 \pm 0.77	47 \pm 7.33	29 \pm 8.54
6	10.27 \pm 0.15	40 \pm 0.33	21 \pm 0.33
7	11.23 \pm 0.29	48 \pm 6.03	36 \pm 6.98

All values are the mean of 3 measurements

In Table 4 there was an improvement in haemoglobin iron and ferritin compare to the baseline in 0 week. The improvement of anaemia parameters were calculated as a percentage in week 7 compared to week 0 was; 123, 202 and 263% for haemoglobin, iron and ferritin respectively. Therefore, level of ferritin achieved the highest percentage.

Table 4: Effect of ingesting aubergines (100g) (*Solanum melongena*) on haemoglobin, iron and ferritin levels among anaemic childbearing Egyptian females

Week	Haemoglobin (g/dl)	Iron (μ g/dl)	Ferritin (μ g/l)
0	9.73 \pm 0.15	29.33 \pm 3.18	17.00 \pm 1.00
1	11.07 \pm 0.23	41.00 \pm 1.15	27.00 \pm 5.69
2	12.43 \pm 0.36	67.00 \pm 5.77	39.00 \pm 3.21
3	10.33 \pm 0.46	44.00 \pm 5.03	29.67 \pm 0.88
4	9.93 \pm 0.35	38.67 \pm 0.88	28.00 \pm 1.00
5	11.23 \pm 0.39	45.67 \pm 6.69	35.00 \pm 3.79
6	10.50 \pm 0.12	37.00 \pm 1.73	28.00 \pm 2.31
7	11.93 \pm 0.18	59.33 \pm 1.76	44.67 \pm 2.33

All values are the mean of 3 measurements

Table 5: Table 1 Effect ingesting black dates (100g) (*Phoenix dactylifera*) on haemoglobin, iron and ferritin levels among anaemic childbearing Egyptian females

week	Haemoglobin (g/dl)	Iron (μ g/dl)	Ferritin (μ g/l)
0	9.53 \pm 0.19	29 \pm 1.86	15 \pm 0.33
1	10.70 \pm 0.30	37 \pm 3.71	18 \pm 0.67
2	11.70 \pm 0.21	58 \pm 8.99	26 \pm 2.91
3	11.07 \pm 0.30	48 \pm 3.61	25 \pm 1.86
4	10.40 \pm 0.38	37 \pm 3.61	23 \pm 1.67
5	11.50 \pm 0.25	45 \pm 2.19	27 \pm 1.76
6	11.37 \pm 0.19	48 \pm 1.15	29 \pm 1.73
7	11.60 \pm 0.21	50 \pm 2.00	31 \pm 2.08

All values are the mean of 3 measurements

In Table 5 there was an improvement in haemoglobin iron and ferritin compare to the baseline in 0 week. The improvement of anaemia parameters were calculated as a percentage in week 7 compared with week 0; 122, 172 and 207% for haemoglobin, iron and ferritin respectively. Therefore level of ferritin achieved the highest percentage compare to other indices

Contrary to our expectations liver results come after plant sources such as aubergine and black dates in improving anaemia indices. Allen, [16] reported that Animal Source Foods (ASF) can provide a variety of micronutrients that are difficult to obtain in adequate quantities from plant source foods alone. Furthermore, ASF provide multiple micronutrients simultaneously, which may be important in diets that are marginally lacking in more than one nutrient. For example, vitamin A and riboflavin are needed for iron mobilization and haemoglobin synthesis and iron supplements may not reduce the prevalence of anaemia if intakes of these other nutrients are low [16]. Thus, foods such as liver that contain substantial levels of both iron and preformed

vitamin A may be more effective than single-nutrient supplements in alleviating poor micronutrient status. Murphy and Allen [17] reported that ASF can fill multiple micronutrient gaps at a lower volume of intake than can plant source foods. Approximately 100 g of cooked beef provides an entire day's recommended intake of protein, vitamin B-12 and zinc and contributes substantially to meeting the riboflavin and iron recommendations. The explanation of the current study disagreement with above results might due to combinations of animal source or presence of vitamin C within the diet consumed. Du and co-workers reported that there are additional factors which may influence iron bioavailability. Supporting to this theory of effects of bioavailability. Theil [18] claimed that the mechanisms of iron uptake into the gut in humans and animals are not known; nor is it known what impact the differences in the plant ferritin mineral (amorphous, high phosphate) and the animal ferritin mineral (crystalline, little phosphorus) have on digestion

Animal Source Foods (ASF) tend to be sources of macronutrients that may not be desirable in the diet, such as saturated fat and cholesterol. ASF also may be undesirably high in total fat, energy and protein. However, the vegetarian diets also tended to be lower in fat and higher in fibre, vitamin C, folate, magnesium, copper and manganese. Du and co-workers [19] were in agreement with our results that showed the prevalence of iron deficiency anaemia (IDA) in rural areas was similar to that in urban areas, although intake of foods from animal sources and heme iron was much lower in rural areas).

Recently, Hunt [20] summarized the studies of iron and zinc status of vegetarians and expressed concern about mineral status for those consuming plant-based diets. The panel setting the new Dietary Reference Intakes for iron assumed 10% iron absorption for vegetarian diets versus 18% absorption for a mixed diet and thus suggested that the Recommended Dietary Allowance for iron should be 80% higher for vegetarians [9].

Results summary

Ferritin: The level of ferritin has achieved the highest level in case of ingesting aubergine, liver, black date, watercress and finally molasses black strap.

Iron: The level of iron has achieved the highest level in case of ingesting aubergine, black date, liver, molasses black strap and finally watercress.

Haemoglobin: The level of haemoglobin has achieved the highest level in case of ingesting aubergine, black date, liver, molasses black strap and finally watercress.

The starkest point in this figure indicates that aubergine increased the levels of anaemia parameters with the highest levels compare to other food items. The hierarchical food that improve anaemia indices were aubergine>black dates>liver>molasses blackstrap>watercress for iron and haemoglobin. Similar results had noticed for ferritin.

DISCUSSION

The researchers have recognised the importance of defining the relationship between iron intake and anemia indices among childbearing Egyptian females. In this study, the results indicated that iron plant sources and liver tissue can enhance levels of serum iron, ferritin and haemoglobin. The results showed that plant sources such as aubergine, black dates improved the level of haemoglobin, iron and ferritin which increases the ratio of haemoglobin, iron and ferritin in serum (Fig. 1). Although the nutritional value of aubergine is poor in terms of iron, about 0.4mg per 100 g but the best results were observed in this source compared to other food items. In fact, there are many factors which might improve the indices of haemoglobin, iron and ferritin. Around 10 to 15 mg of iron are ingested per adult/day. Around 10-20% must be absorbed to match "obligatory" losses. However, humans have no mechanism for iron excretion so homeostasis occurs through the control of absorption. This can fail due to genetic disturbances and lead to either excessive iron uptake or poor iron absorption and, thereby, deficiency. The latter problem is mostly due to a lack of absorbable dietary iron most commonly outside the Western world and almost exclusively due to poor dietary intakes [21]. There are different factors that would increase the iron absorption from non-heme foods:

- A good source of vitamin C (ascorbic acid)-i.e., oranges, grapefruits, tomatoes, broccoli and strawberries, eaten with a none-heme food.
- A heme and non-heme food eaten together.
- A non-heme food cooked in an iron pot, such as a cast iron skillet.

One of the main aims in this work was to develop strategies to select right foods and thereby improve their nutritional quality of plant iron intake. The study focuses on the plant nutritional biochemistry of essential human nutrient; iron and to provide examples of the type of foods that can help improving nutritional anaemia among childbearing Egyptian females. However, food habits can determine the bioavailability of iron, as

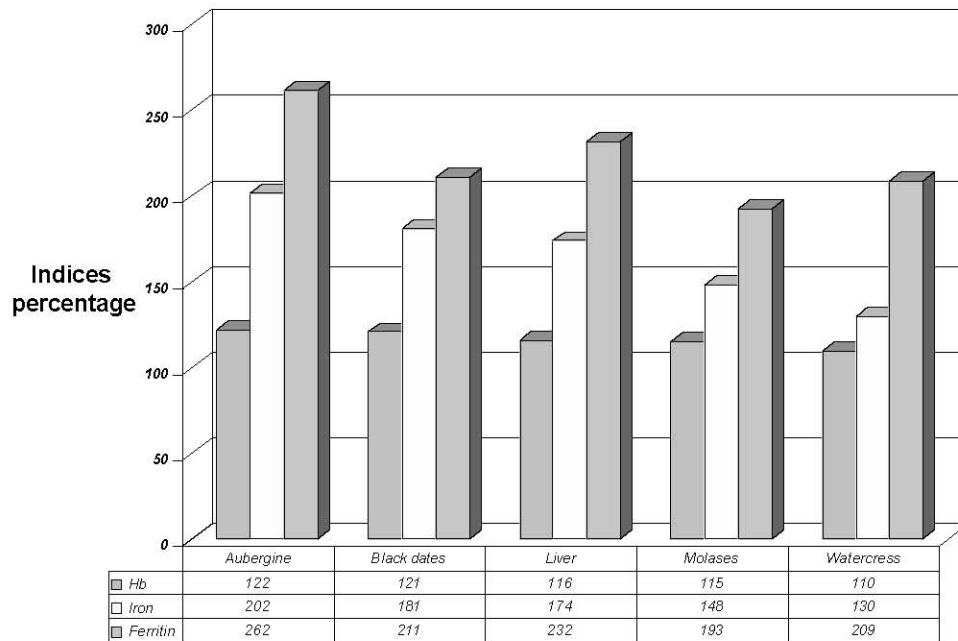


Fig. 1: The relationship between anaemia indices and type of ingested food

explained previously. The results of current work were consistent with Du and co-workers [19] and inconsistent with Murphy and Allen [17]. As explained previously. We also showed that poor iron status is associated with lower socio-economic status and that females are at particular risk of low iron status.

Iron intakes and status of childbearing Egyptian females: Overall, we have shown that iron status (indicated by blood haemoglobin, serum ferritin and other blood-based indices) is not associated with total iron intake but is generally related positively with meat and fruit consumption (promoters of iron absorption), particularly adolescent girls. With a view to making recommendations and defining future research needs.

CONCLUSIONS

The results of this study indicate that plant iron sources such as aubergine, black dates, molasses blackstrap and watercress can improve iron, haemoglobin and ferritin levels among childbearing Egyptian females. Iron plant sources are less bioavailable but there are different factors that would increase the iron absorption from non-heme foods. Poor iron status is associated with lower socio-economic status among childbearing Egyptian females.

REFERENCES

1. Dallman, P.R., 1986. Biochemical basis for the manifestations of iron deficiency. *Ann. Rev. Nutr.*, 6: 13-40.
2. Institute of Medicine, 2001. Food and Nutrition Board. Dietary Reference Intakes for Vitamin A, Vitamin K, Arsenic, Boron, Chromium, Copper, Iodine, Iron, Manganese, Molybdenum, Nickel, Silicon, Vanadium and Zinc. National Academy Press, Washington, DC.
3. Andrews, N.C., 1999. Disorders of iron metabolism. *New England J. Med.*, 341: 1986-1995.
4. Bothwell, T.H., R.W. Charlton, J.D. Cook and C.A. Finch, 1979. *Iron Metabolism in Man*. Blackwell Scientific St. Louis, Oxford
5. Bhaskaram, P., 2001. Immunobiology of mild micronutrient deficiencies. *Br. J. Nutr.*, 85: S75-S80.
6. Haas, J.D. and T. Brownlie, 2001. 4th. Iron deficiency and reduced work capacity: a critical review of the research to determine a causal relationship. *J. Nutr.* 131: 691S-696S.
7. Corbett, J.V., 1995. Accidental poisoning with iron supplements. *Am. J. Mater. Child Nursing*, 20: 234.
8. World Health Organization, 1992. National strategies for overcoming micronutrient malnutrition. Agenda item 21, 45th World Health Assembly, (unpublished).

9. World Health Organization, 1992. The prevalence of anemia in women: A tabulation of available information. Geneva, (document WHO/MCH/MSM/92.2).
10. Grusak, M.A., 2006. Plant sources of dietary iron: Diversity in tissue iron concentration. In: Proceedings of the Thirteenth International Symposium on Iron Nutrition and Interactions in Plants, July 3-7, Montpellier, France.
11. U.S. Department of Agriculture, Agricultural Research Service. 2003. USDA Nutrient Database for Standard Reference, Release 16. Nutrient Data Laboratory Home Page, <http://www.nal.usda.gov/fnic/foodcomp>.
12. Dreux, C., 1977. *Annales de Biologie Clinique*, 35: 275.
13. Bemard, A. and R. Lauwerys, 1984. Turbidimetric latex immunoassay for serum ferritin. *J. Immunol. Methods*, 71: 141-147.
14. Van Kampen, E.J. and W.G. Zijlstra, 1961. Standardization of hemoglobinometry II. The hemiglobincyanide method. *Clinica Chimica Acta*, 6: 538-544.
15. International committee for standardization in haematology 1967. *Br. J. Haematol.*, 13 (Supp) 71.
16. Allen, L.H., 2002. Iron supplements: scientific issues concerning efficacy and implications for research and programs. *J. Nutr.*, 132: 813S-819S.
17. Murphy, P.S. and H.L. Allen, 2003. Supplement: Animal Source Foods to Improve Micronutrient Nutrition in Developing Countries Nutritional Importance of Animal Source Foods. *J. Nutr.*, 133: 3932S-3935S.
18. Theil, E.C., 2003. Ferritin: At the Crossroads of Iron and Oxygen Metabolism. Supplement: 11th International Symposium on Trace Elements in Man and Animals. *J. Nutr.*, 133: 1549S-1553S.
19. Du, S., F. Zhai, Y. Wang and M.B. Popkin, 2000. Current methods for estimating dietary iron bioavailability do not work in China. *J. Nutr.*, 130: 193-198.
20. Hunt, J.R., 2002. Moving toward a plant-based diet: Are iron and zinc at risk? *Nutrition Review*, 60: 127-134.
21. MRC Human Nutrition Research, <http://www.mrc-hnr.cam.ac.uk/research/micronutrients/iron.html>.