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Contenido

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Contents

Las opiniones expresadas en las presentaciones y discusiones sostenidas durante este Simposio y presentadas en este documento representan solamente las opiniones de sus respectivos autores y no necesariamente los puntos de vista de las organizaciones a las que están afiliados o las entidades patrocinantes.

Prefacio

El Simposio Dr. Miguel Layrisse, Hierro y Nutrición: Conocimientos Actuales se llevó a cabo el 16 de septiembre de 1999 en Caracas, Venezuela como parte del programa científico del Congreso de Pediatría de la Sociedad Venezolana de Puericultura y Pediatría. Este evento científico se organizó en reconocimiento a sus contribuciones al conocimiento actual sobre el hierro y nutrición a lo largo de su fructífera carrera científica. Este Simposio es una iniciativa de Kellogg´s con el apoyo de instituciones reconocidas como el Instituto Venezolano de Investigaciones Científicas, la Sociedad Latinoamericana de Nutrición, la Fundación Cavendes, el Instituto Nacional de Nutrición - Venezuela, la Sociedad Venezolana de Puericultura y Pediatría e ILSI. En este Simposio los expertos nacionales e internacionales revisaron los conocimientos actuales de nutrición de hierro de manera crítica. El evento sirvió de foro para la discusión entre nutricionistas, pediatras, investigadores y expertos de instituciones académicas, industria y gobierno. Esta actualización en el área de nutrición y hierro es propicia toda vez que a pesar de las múltiples estrategias que se llevan a cabo desde hace ya varios años, persisten cifras inaceptablemente altas de prevalencia de deficiencia de hierro y anemia en la población latinoamericana.

Esperamos que los lectores encuentren en este Suplemento un resumen útil de los aspectos más relevantes de la nutrición de hierro y puedan hacer uso de la información científica aquí discutida para tomar acciones concretas y lograr mejorar las condiciones de vida de nuestros niños y niñas de Latinoamérica.

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HIERRO Y NUTRICION: CONOCIMIENTOS ACTUALES

Memorias del Simposio Dr. Miguel Layrisse
realizado en Caracas, Venezuela
16 de Septiembre de 1999

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Deficiencia de hierro hacia el año 2000

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RESUMEN. Este es un resumen no exhaustivo de los últimos 50 años sobre la evolución del metabolismo del hierro y de lo que disponemos en la actualidad para el diagnóstico de la deficiencia de hierro y sus efectos sobre la salud. En los años cuarenta la absorción del hierro se practicaba por determinación química. La cantidad de hierro absorbido se calculaba por la diferencia entre la cantidad de hierro ingerido y la cantidad excretada en las heces. El otro método que se usaba para medir la absorción del hierro de la alimentación era la repleción de la hemoglobina. En la década del 70 se señala como importante contribución al metabolismo del hierro la medida de la concentración de ferritina en el plasma para evaluar la deficiencia de hierro y sobrecarga de hierro. Esa misma década marcó para la ciencia del metabolismo del hierro una etapa de avance importante debido al resultado del marcado extrínseco e intrínseco de los alimentos. Los años 70 y 80 se caracterizaron también por la búsqueda de los inhibidores de la absorción del hierro destacándose los efectos inhibidores del café y el calcio, los del té, del zinc y la fibra. La década del 80 y 90 de caracterizaron además por los conocimientos acerca de la absorción del hierro de un alimento, de una comida y de una dieta completa y por el efecto favorable de la fortificación de los alimentos con hierro en los países en vías de desarrollo; también se estudio el efecto del exceso de hierro del organismo para la salud en general, y el infarto del miocardio en particular en países desarrollados.
Palabras clave: Hierro, absorción de hierro, metabolismo del hierro.

SUMMARY. Iron deficiency towards the new millenium. This is a non-comprehensive overview of the latest 50 years about the evolution of iron metabolism and the methodology we currently have for the diagnosis of iron deficiency and its effects on human health. In the 40's iron absorption was determined by chemistry. The amount of iron absorbed was calculated as the difference between dietary iron and excreted iron. The other methods used to measure dietary iron was hemoglobin repletion. In the 70's the measurement of plasmatic ferritin was an important contribution to iron metabolism to assess iron deficiency and iron overload. In the same decade the extrinsic and intrinsic labelled methodology was an important advancement. The 70's and 80's were years where scientists aimed at finding iron absorption inhibitors, namely coffee, calcium, tea, zinc and fiber. The 80's and 90's were characterized for the emerging knowledge an iron absorption from a food, a meal and a complete diet and for the favorable effect of food iron fortification in developing countries. Also for the effect of iron excess in overall health and miocardial infarction in developed countries were studied.
Key words: Iron, iron metabolism, iron deficiency, iron absorption.

Voy a comenzar esta charla con la década del cuarenta, época en que me gradué de Doctor en Medicina y comencé a interesarme en la hematología, obteniendo una beca de la Universidad Central de Venezuela en 1949 para estudiar esa especialidad en el New England Medical Center de Boston bajo la dirección del Dr. William Damashek. En esa década la absorción del hierro se practicaba por determinación química. Los sujetos en estudio se sometían a una dieta por dos ó tres semanas, hospitalizados en una clínica, colectando todas las heces. La cantidad de hierro absorbido se calculaba por la diferencia entre la cantidad de hierro de la dieta y la cantidad excretada. Ya se puede imaginar la pericia de los investigadores para establecer la absorción que era entre 5% y 10% de la ingesta (1), 1 mg en el hombre y 1,5 mg en la mujer por día.

El otro método que se usaba para medir la absorción del hierro de la alimentación era la repleción de la hemoglobina. Primero se sangraba al sujeto hasta cierta concentración de hemoglobina, se alimentaba con la dieta a estudiar y se esperaba un tiempo prudencial para que concentración de hemoglobina volviera a su valor original. La diferencia entre las dos concentraciones de hemoglobina multiplicado por el volumen total de sangre circulante y dividido por el número de días que duró el experimento estimaba la absorción diaria de hierro de la dieta.

En 1951 Moore and Dubach publicaron los primeros estudios de absorción de hierro de vegetales utilizando cultivos hidropónicos de plantas a las cuales se le inyectaba hierro radioactivo (2). En 1946, los mismos autores ya habían publicado la utilización de inyección intravenosa de hierro radioactivo en conejos para obtener hemoglobina marcada y así poder determinar la absorción del hierro de este compuesto (3).

En 1959 asistí a una reunión de la Organización Mundial de la Salud donde presenté los resultados de prevalencia de anemia por deficiencia de hierro y de folato en el embarazo. Entre los investigadores presentes en esa reunión estaba el Dr. Clement Finch quien me sugirió la posibilidad de colaborar con su laboratorio en Seattle en el estudio de absorción de hierro de los alimentos utilizando los habitantes de esas poblaciones rurales en las cuales la prevalencia de deficiencia de hierro era muy alta debido principalmente a las infecciones por anquilostomo.

Durante la década del 60 trabajé con muestras vegetales de cultivos hidropónicos que me enviaba el Dr. Walker desde Seattle. En el laboratorio del Instituto Venezolano de Investigaciones Científicas preparamos cultivos hidropónicos para los frijoles negros y maíz e inyectamos hierro radioactivo a ternera, conejos y peces de agua salada (pargo) para obtener carne de ternera, pescado y hemoglobina .

Estos estudios finalizados en 1968 fueron publicados en Blood 1969 (4), comprendían 131 estudios de absorción de hierro en harina de trigo, harina de maíz, frijoles negros, lechuga, espinaca, soya, carne de ternera, hemoglobina y pescado.

En esa década también colaboré con los doctores Bothwell y Finch en la determinación de la excreción de hierro del cuerpo humano (5). Dicho estudio fue clave para determinar con exactitud la cantidad de hierro excretado, la cual fue de 0.90 por día en hombres de raza blanca de los Estados Unidos y mestizos de Venezuela en comparación con resultados anteriores que señalaban varios miligramos/día.

A finales de esa década publiqué el efecto de interacción de varios alimentos en la absorción del hierro demostrando que la absorción del músculo de ternera y la hemoglobina eran diferentes en absorciones separadas pero iguales cuando se administraba en la misma comida (6). Estos estudios fueron el punto de partida del marcado intrínseco y extrínseco de los alimentos y los conceptos de los pools de hierro hemínico y no-hemínico, que luego se estudiaron con mas detalle en la siguiente década.

También derivado de los estudios efectuados por los doctores Roche y Layrisse en esa década se publicó un folleto sobre la naturaleza y causa de la infección por anquilostomo (7) y también por primera vez la reducción de la sobrevida de los glóbulos rojos en la anemia por deficiencia de hierro severa (8) asociados y no asociados con la infección por anquilostomo.

La década del 70 marcó para la ciencia del metabolismo del hierro una etapa de avance importante debido al resultado de marcado extrínseco e intríseco de los alimentos; el primer estudio fue llevado a cabo en colaboración de los laboratorios de la Universidad de Washington en Seattle y el Instituto Venezolano de Investigaciones Científicas, y consistió en agregarle hierro radioactivo (Fe^{55}) a cultivos hidropónicos de vegetales y luego agregarle una sal de hierro marcada con ^{59}Fe, la consistente relación entre los dos isótopos cercana a la unidad indica la factibilidad de estudiar la absorción del hierro no-hemínico utilizando el marcado extrínseco. La misma relación se obtuvo de una comida de varios alimentos. Resultado de ese experimento fue el punto de partida de otros experimentos en los cuales se utilizaba el marcado extrínseco de los alimentos.

Durante esa década varios investigadores estudiaron la absorción del hierro de dietas locales utilizando el marcado extrínseco de los alimentos entre ellos se señalan a Cook, Hallberg y Monsen (1). Igualmente varios investigadores han estudiado la absorción del hierro de alimentos fortificados con varios compuestos de hierro. Entre otros, se demostró que el hierro EDTA tenía una absorción dos ó tres veces mayor que el hierro del sulfato ferroso. En uno de los estudios se demostró que los fitatos de los cereales tienen poco efecto inhibidor sobre la absorción de este compuesto.

La deficiencia de hierro produce un cambio en la salud, tanto mental como física y ha tomado espacio en la década del 70 y en las siguientes décadas. Desde los estudios de Viteri y Garby sobre la capacidad para el trabajo, seguidos por los trabajos de Pollit, Lozoff, Oski, Walter, Scrimshaw y recientemente con Beard sobre el desarrollo psicomotor y mental (1).

La Compañía Kellogg's han publicado recientemente un folleto sobre el particular titulado "Efectos de la anemia en la deficiencia de hierro en el desarrollo mental y motor y su comportamiento en niños".

Durante esa década varios investigadores estudiaron la absorción del hierro de dietas locales utilizando el marcado extrínseco de los alimentos.

En la década del 70 se señala como importante contribución al metabolismo del hierro la medida de la concentración de ferritina en el plasma para evaluar la deficiencia de hierro y sobrecarga de hierro. La determinación de ferritina en el plasma se realizó primero por radio inmunoensayo y después por ELISA tipo sandwich. Se ha estimado que por cada µg/L de ferritina en el plasma hay 8 mg de hierro de reserva.

A la contribución relevante del Dr. James Cook sobre el metabolismo del hierro hay que agregar los estudios sobre el receptor de la transferrina en el suero, el cual es un indicador para evaluar la deficiencia de hierro que aumenta en la medida que disminuyen las reservas de hierro (9).

La década del 70 y 80 se caracterizó también por la búsqueda de los inhibidores de la absorción del hierro destacándose los efectos inhibidores del café y el calcio por los estudios del Dr. Cook, los del té por el Dr. Bothwell y del zinc y la fibra por el Dr. Hallberg (1).

Durante las dos ultimas décadas es oportuno mencionar el estudio del Dr. Viteri (10) y colaboradores sobre el tratamiento de la anemia por deficiencia de hierro sustituyendo la dosis diaria de hierro por una dosis semanal o dos veces a la semana, la cual produce el mismo efecto sobre el incremento de la concentración de hemoglobina y reduce considerablemente los trastornos gastrointestinales producidos por las sales de hierro. El Dr. Viteri ampliará con mas detalles sobre el nuevo

tratamiento de la anemia por deficiencia de hierro.

La década del 80 y 90 de caracterizó además de la absorción del hierro de un alimento, de una comida y de una dieta completa y por el efecto favorable de fortificación de los alimentos con hierro en los países en vías de desarrollo; y por otra parte el efecto del exceso de hierro del organismo sobre la salud en general, y el infarto del miocardio en particular en países desarrollados. Hay 4 ejemplos muy demostrativos de la fortificación de los alimentos. Garby y Arelkel reportaron el efecto de la fortificación de salsa de pescado con hierro EDTA en Tailandia. Después de un año de fortificación el hematocrito de la sangre periférica aumentó 1.5% comparado con el grupo control (11). Viteri y colaboradores en tres poblaciones de América Central, demostraron que el enriquecimiento del azúcar con hierro-EDTA producía un aumento significativo de la ferritina en el plasma 20 meses después de iniciada la fortificación (12). En Africa del Sur el grupo de investigadores dirigido por el Dr. Bothwell fortificaron con hierro-EDTA el curry de una comunidad Indú, obteniendo después de un año un aumento significativo de la concentración de hemoglobina y la ferritina plasmática (13). Finalmente, la experiencia venezolana demuestra que la fortificación de la harina de maíz y trigo con fumarato ferroso y vitamina A, Tiamina, Rivoflavina y Niacina reduce la prevalencia de anemia y deficiencia de hierro de 37% y 19% a 15% y 10% respectivamente, después de un año de fortificación (14).

Esa reducción tan dramática de la anemia y deficiencia de hierro motivaron estudios posteriores que demostraron que la vitamina A y ß-caroteno previenen el efecto inhibidor sobre la absorción del hierro que producen los fitatos y polifenoles contenidos en los alimentos. Aparentemente la vitamina A se une al hierro en la digestión y actúa como un quelante previniendo el efecto inhibidor de los fitatos y polifenoles (15-17).

Con respecto al exceso de hierro, en 1992 se encontró una relación significativa entre la concentración de ferritina en el suero y el riesgo de contraer infarto del miocardio (2.64 veces, $p<0.001$) (18). Otros autores han sugerido que el límite de riesgo de sufrir infarto del miocardio es de 400 µg/L en hombres, 300 µg/L en las mujeres en edad fértil y 200 µg/L en las menopáusicas (19). La teoría oxidativa del estrés es la más sustentada por los autores que han escrito sobre el tema. Sin embargo, las encuestas epidemiológicas recientes no respaldan los trabajos de los Finlandeses, de considerar los altos niveles de ferritina como un factor de riesgo para desarrollar infarto del miocardio (20).

Este es un resumen no exhaustivo de los últimos 50 años sobre la evolución del metabolismo del hierro y de los que disponemos en la actualidad para el diagnóstico de la deficiencia de hierro y los efectos mentales y físicos en la salud. Los oradores siguientes suministrarán nuevos conocimientos sobre los diversos aspectos mencionados en mi charla.

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The nutritional assessment of iron status

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SUMMARY. In nutritional studies to assess the prevalence of iron deficiency, it has been common practice to define 3 stages of increasing severity: iron storage depletion as defined by a low serum ferritin, mild iron deficiency without anemia based on laboratory evidence of iron deficient erythropoiesis (IDE), and overt iron deficiency anemia (IDA). While this approach provides a broad perspective of impaired iron status, the main liabilities of iron lack are associated only with the more advanced stage of IDA. Consequently, the hemoglobin determination can be used to screen for nutritionally significant iron deficiency. Having identified anemia, more specific laboratory studies are needed to establish iron lack as the cause. The traditional measurements of iron deficient erythropoiesis (IDE) such as a low transferrin saturation, elevated erythrocyte protoporphyrin, or decreased mean corpuscular volume are commonly used. The major drawback in using these parameters is that they are affected similarly in individuals with the anemia of chronic disease (ACD), a common form of anemia in low socioeconomic populations. Because iron stores are invariably absent in individuals with uncomplicated IDA, a low serum ferritin concentration below 20 µg/L confirms the diagnosis of IDA when anemia is present. The main limitation of the serum ferritin is that it is falsely elevated to within the normal range when IDA develops in individuals with concurrent infection or chronic inflammation. When this occurs in a clinical setting, a bone marrow examination is commonly performed to identify IDA. Recent investigations indicate that this cumbersome procedure can be avoided by measuring an important new iron-related measurement, the serum transferrin receptor (TfR). Because the synthesis of TfR is upregulated with tissue iron deficiency, IDA can be identified readily by an elevated serum TfR. Importantly, the serum TfR is normal in individuals with the ACD but becomes elevated if these individuals develop IDA. The optimal combination of laboratory measurements for detecting IDA is the hemoglobin, serum ferritin and serum TfR.
Keywords: Iron deficiency anemia, ferritin, protoporphyrin, ànemia, iron deficiency, assesment, iron status.

RESUMEN. Evaluación del estado nutricional de hierro. En estudios nutricionales para evaluar la deficiencia de hierro, se han definido comunmente tres estadios de severidad creciente: agotamiento de las reservas de hierro, definido por bajos niveles de ferritina sérica, deficiencia de hierro moderada sin anemia basada en evidencia de laboratorio de eritropoyesis deficiente en hierro (EDH) y anemia por deficiencia de hierro (ADH). Aunque esta clasificación provee una amplia perspectiva de la alteración de estado de hierro, las principales consecuencias de la falta de hierro están asociadas con estadíos más avanzados de anemia por deficiencia de hierro. Consecuentemente, la determinación de hemoglobina puede usarse para tamizar la deficiencia de hierro nutricionalmente significativa. Una vez identificada la anemia, estudios de laboratorio más específicos son necesarios para establecer la falta de hierro como causa. Las mediciones tradicionales de eritropoyesis deficiente en hierro (EDH) tales como una baja saturación de transferrina, elevada protoporfirina eritrocitaria, o la disminución del volumen corpuscular medio se utilizan comúnmente. La principal desventaja de estos parámetros es que éstos están afectados de manera similar en individuos con la anemia de enfermedades crónicas, una forma común de anemia en poblaciones de bajo nivel socioeconómico. Debido a que las reservas de hierro se encuentran ausentes en la anemia por deficiencia de hierro sin complicaciones, una concentración de ferritina sérica por debajo de 20 µg/L confirma el diagnóstico de anemia por deficiencia de hierro una vez que la anemia está presente. La principal limitación de la ferritina sérica es que puede encontrarse falsamente elevada o en límites normales cuando la anemia por deficiencia de hierro se desarrolla en individuos con infección o inflamación crónica concurrente. Cuando esto ocurre en el medio clínico, un examen de médula ósea se realiza comúnmente para identificar la ADH. Las investigaciones recientes indican que este procedimiento puede evitarse midiendo un nuevo indicador relacionado al hierro llamado receptor sérico de transferrina (RTf). Debido a que la síntesis de RTf está regulada paralelamente con la deficiencia de hierro tisular, la ADH puede identificarse rápidamente por niveles elevados de RTf. Es importante destacar que el RTf es normal en individuos con anemia por enfermedades crónicas pero aumenta si estos individuos desarrollan ADH. La combinación óptima de las mediciones de laboratorio para detectar la ADH son la hemoglobina, la ferritina sérica y el RTf sérica.
Palabras clave: Deficiencia de hierro, evaluación, estado nutricional, ferritina, protoporfirina, anemia.

INTRODUCTION

Reliable laboratory methods to evaluate iron status are of critical importance in efforts to improve the iron nutrition of a population. In countries where iron deficiency is less common, iron measurements are needed to assess prevalence rates in susceptible segments of the population and to monitor changes in iron status over time. In developing countries where iron deficiency is more prevalent, reliable measurements of iron status play an important role in the assessment of new intervention strategies. The iron measurements that are suitable for epidemiological studies have undergone a continual refinement during the past several decades and newer methods with greater specificity and sensitivity for identifying iron deficiency have been added. There are several considerations when selecting iron-related methods such as cost, suitability for capillary blood sampling, ease of laboratory performance and adaptability for field studies. There is no single method or combination of methods that is satisfactory for all purposes. The following discussion of available iron measurements will emphasize the measurement of serum transferrin receptor, an important new method for assessing iron status.

Definitions of iron status

The initial stage of iron lack is storage iron depletion in which the continuous supply of iron for hemoglobin production is adequate but no buffer of body iron reserves exists to cover short-term needs. The only practical method for identifying this early stage of iron deficiency in population studies is the serum ferritin concentration that varies directly with iron stores in otherwise normal individuals (1,2). Values below 10-20 μg/L indicate absent iron stores. The main limitation of the serum ferritin is that chronic infection or inflammation elevates the concentration 2-3 times higher than values representing iron stores (3). In developing countries where inflammatory diseases are more common, cut-off levels of 30 μg/L or higher have been used but these modified definitions should be established on the basis of bone marrow examinations or therapeutic iron trials (4).

A curtailment in the supply of transferrin-bound iron to developing red blood cells is referred to as iron deficient erythropoiesis (IDE). The serum iron, total iron-binding capacity (TIBC), and the transferrin saturation calculated as the ratio of serum iron/TIBC are the traditional indices of IDE. Unfortunately, these iron transport measurements are affected by numerous physiological and pathological processes and consequently have low specificity for identifying iron deficiency as the cause of IDE. Their major value is in excluding iron deficiency as the cause of anemia when the transferrin saturation is normal or increased. An elevated free erythrocyte protoporphyrin is more specific for iron deficiency but this measurement is increased with excess lead exposure as well as in the anemia of chronic disease (ACD). Microcytosis of circulating red blood cells as reflected by a low mean corpuscular volume is a useful index of IDE, but like most other laboratory measurements, it does not distinguish iron deficiency from inflammatory diseases.

The most advanced stage of iron lack is iron deficiency anemia (IDA), the severity of the anemia reflecting the degree of iron lack. Few, if any, of the nutritional consequences of iron deficiency such as decreased learning capacity in infants, impaired work performance in adults, and increased perinatal morbidity and mortality have been demonstrated in absence of anemia. Consequently, the hemoglobin concentration is an indispensible index of the impact of iron deficiency on health and well-being. The hemoglobin concentration should not be the only index of iron status because there are numerous diseases and deficiency states that result in anemia. One of the key laboratory measurements that is often used in tandem with hemoglobin to identify IDA is the serum ferritin assay. The combination of hemoglobin and serum ferritin measurements was used in a highly effective manner by Layrisse and coworkers to assess the efficacy of a large-scale program of food iron fortification in Venezuela (5). However, as discussed previously, falsely normal or elevated levels due to chronic inflammation diminish the utility of using only the serum ferritin and hemoglobin to identify IDA. The development of the serum transferrin receptor (sTfR) assay has circumvented many of the problems in identifying IDA in populations where infections and IDA are common.

Serum Transferrin Receptor

The movement of iron within the body is controlled by a specific membrane receptor for transferrin iron that varies in amount with the iron needs of the cell. The presence of a circulating form of this protein was first reported by a group of Japanese workers (6). Later investigators demonstrated that the serum form is a soluble fragment containing most of the large extracellular domain of the transferrin receptor (7). There have been several extensive reviews of this new iron-related measurement (8-10). The concentration of the serum transferrin receptor (sTfR) is directly proportional to the total body mass of transferrin receptor, 80% of which is derived from the red cell precursors in the bone marrow. There are only two conditions that elevate the concentration of the sTfR - an increase in red cell precursors in the bone marrow (erythropoiesis) and tissue iron deficiency. There are many forms of anemia that are associated with enhanced erythropoiesis but these are not encountered frequently enough in population studies to diminish the usefulness of the sTfR for identifying IDA.

Serial phlebotomy studies in normal adults have shown that the sTfR concentration remains normal during the progressive depletion of iron stores (11). With the onset of tissue iron deficiency, the sTfR concentration increases in direct proportion to the severity of the deficiency. Because of the reciprocal changes in ferritin and sTfR at varying levels of body iron, the ratio of the logarithm of the receptor/ferritin

ratio bears a precise inverse relationship to body iron levels over a wide range from iron repletion to advanced IDA (11). This observation is hardly surprising in view of the tight reciprocal regulation of the synthesis of these two key iron proteins by means of the iron regulatory proteins (12). When the supply of iron to tissues is adequate, ferritin synthesis is upregulated to store any excess of intracellular iron. When the supply of iron to body tissues is inadequate, ferritin synthesis is reduced and the synthesis of transferrin receptor is enhanced to allow the cell to compete more effectively for the transferrin-bound iron in its environment.

The laboratory methods for measuring the sTfR are similar to the sensitive immunoassays that are used to assay the serum ferritin, differing only in regard to the immunological reagents. There are number of assays for the sTfR that are available commercially but, unfortunately, there is a wide range of reported normal values. The urgent need for standardization of sTfR assays has been repeatedly emphasized in the published literature (13). One important advantage of using the sensitive immunological assays of ferritin and sTfR is that only a few microliters of plasma or serum is required. In a recent investigation, ferritin and receptor measurements were performed on capillary blood spotted onto filter paper and allowed to dry (14). The sTfR results were identical to the spotted samples after correction was made for displacement of serum by red cells. However, the spotted ferritin values were significantly higher than serum ferritin values due to the release of ferritin from hemolyzed red blood cells. This problem was circumvented in part by using the receptor/ ferritin ratio of the spotted blood samples that proved to be as reliable as serum determinations for distinguishing normal subjects from those with IDA. The distinction between milder iron deficiency without anemia and either normal or IDA was less satisfactory with spotted samples than with serum. Recent studies have shown that the latter difficulty can be circumvented by using spotted capillary plasma samples rather than whole blood (15). However, in certain field conditions, the requirement for centrifugation of samples could be difficult. The use of capillary blood samples to prepare either whole blood or serum paper spots offers a significant advantage in field surveys by eliminating the need for venous sampling and thereby simplifying the storage and transport of specimens.

As mentioned previously, one problem in assessing iron status in lower socioeconomic segments of a population is the difficulty in distinguishing IDA from the ACD. The latter form of anemia, which is associated with a wide spectrum of chronic inflammatory or infectious disorders, is encountered frequently in clinical practice. The pathogenesis of this common anemia is still unclear (16,17). In one study in anemic patients above 65 years of age, 36% had IDA while 44% had ACD based on bone marrow examinations, the conventional method of making this distinction (18). Recent clinical studies indicate that this cumbersome procedure can be circumvented by measuring the sTfR.

An initial evaluation of the sTfR in anemic patients with inflammatory illnesses such as rheumatoid arthritis or chronic bacterial infections demonstrated that the sTfR remains normal in contrast to the distinct elevation in patients with IDA (19).

In a subsequent report, 129 anemic patient underwent bone marrow examinations to assess their iron status (20). The three diagnostic categories included 48 patients with IDA, 64 with the ACD, and 17 with both IDA and the ACD. The best separation between the groups was obtained with the ratio of receptor/ferritin that distinguished between patients with IDA and ACD and with a single exception, between those with ACD and both IDA and ACD. The key finding in this study was that the ratio of receptor/ferritin can be used to identify IDA even in the presence of inflammatory disease.

The utility of the sTfR is not limited to distinquishing IDA from other forms of anemia. Recent investigations have demonstrated its usefulness in the assessment of milder iron deficiency without anemia (21,22). These studies support the earlier work of Skikne et al. who demonstrated that the sTfR becomes elevated during the progression of iron deficiency well in advance of developing overt IDA (11).

CONCLUSIONS

Several recent clinical investigations indicate that tandem measurements of serum ferritin and sTfR offer major advantages in identifying IDA in population studies. These measurements have the practical advantage of requiring only a small sample of capillary blood and thereby permitting the transport and storage of samples on filter paper.

A significant limitation of the sTfR is poor standardization between laboratories and commercially available assay kits. In addition, more information is needed about the reliability of the receptor/ferritin index in the presence of other nutrient deficiencies such protein, vitamin A, folic acid, or vitamin B_{12}. Despite these limitations, the initial experience with coupled measurements of serum ferritin and sTfR suggest that this approach is a meaningful advance in our ability to assess the nutritional iron status of a population.

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Iron supplementation as a strategy for the control of iron deficiency and ferropenic anemia

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SUMMARY. Iron supplementation is a public health strategy designed for the prevention of iron deficiency and its consecutive anemia. It should be targeted, safe, flexible, long term and ideally, community based under the supervision of the health sector. It must be differentiated from iron therapy, even though, in the intermediate and long term it corrects mild-moderate deficiency of iron and ferropenic anemia. It should complement other measures for the control of iron deficiency.

A summary of results comparing daily and intermittent iron supplementation (every 3-days in rats, and weekly in humans) is presented, including studies in an animal model, human supplementary-iron absorption studies, clinical research and field studies. It is concluded that intermittent iron supplementation is efficacious and, that in the long term it achieves an increase in iron reserves while avoiding sustained oxidative stress caused by current practices of excess daily iron supplementation, particularly in the developing world. The stage is set for long-term weekly iron supplementation programs in large population groups to determine its sustainability and effectiveness.

Keywords: Iron, supplementation, daily supplementation, weekly suplementation, efficacy, safety, oxidative stress.

RESUMEN. La suplementación con hierro como estrategia para el control de la deficiencia de hierro y la anemia ferropenica. La suplementación con hierro es una estrategia de salud pública diseñada para la prevención de la deficiencia de hierro y la anemia consecuente. Esta estrategia debe ser dirigida, segura, flexible, a largo plazo e idealmente realizada en la comunidad con supervisión del sector salud. Debe estar diferenciada de la terapia con hierro, aunque a mediano y largo plazo, esta estrategia corrige la deficiencia leve-moderada de hierro y la anemia ferropenica. Debe complementarse con otras medidas de control de la deficiencia de hierro. Se hace una revisión de los resultados de estudios que comparan la suplementación diaria e intermitente (cada 3 días en ratas, y semanal en humanos), incluyendo estudios en modelos animales, estudios de absorción de hierro suplementado en humanos, estudios clínicos y estudios de campo. Se concluye que la suplementación intermitente es eficaz y que a largo plazo logra el aumento de las reservas de hierro evitando el estres oxidativo sostenido causado por las prácticas actuales de suplementación diaria, particularmente en países en desarrollo. La plataforma está lista para determinar la sostenibilidad y efectividad de programas a largo plazo de suplementación con hierro semanalmente en grupos poblacionales grandes.

Palabras clave: Hierro, suplementación, suplementación diaria, suplementación semanal, eficacia, seguridad, estres oxidativo.

The concept of iron supplementation

According to Websterís dictionary, a "supplement is something added especially to make up for a lack or deficiency". Applied to public health nutrition and to iron in particular, the concept of supplementation consists in providing a defined amount of the mineral in a pharmaceutical preparation that can be consumed with or apart from meals, with the purpose of safely bringing the total utilizable iron to a level considered optimal for the health of individuals and populations.

The following is implied in that definition:

1- That an iron supplement should be given when the total dietary iron intake from native and/or fortified foods fails to reach that desired level in individuals or populations.
2- That its primary purpose is preventive rather than therapeutic of an established iron deficiency. In the long term it should correct mild-moderate deficiencies.
3- From the above considerations, it is obvious that supplementation should be targeted to vulnerable populations or groups at risk of developing iron deficiency.
4- That it should be integrated with other measures aimed at controlling iron deficiency (and ideally at controlling other nutritional deficiencies). Therapeutic iron, when indicated, should be included among other control measures.
5- That as with any other intervention, it should be monitored and evaluated, and should be flexible to the point of discontinuing it when considered no longer necessary.

Just as I have stated what iron supplementation should be or include, I would like to present what it is not or should not be:

1- It should not be considered a therapeutic intervention aimed at correcting ferropenic anemia in particular, and much less anemia in general, in a short period of time.

2- It should not be seen as an intervention that excludes all others aimed at controlling iron deficiency: food-based strategies, infection control measures, iron therapy, etc., but rather as a complement to these other measures.

Brief historical background

Iron supplementation is an off-shoot of iron therapy and has been applied almost exclusively to the correction and prophylaxis of ferropenic anemia in pregnancy. After many publications and several conferences on the clinical management of iron deficiency (e.i.1). The World Health Organization (WHO) started a series of studies of gestational anemia in India (2), and in 1972 it convened a group of experts (3) which concluded that vulnerable segments of the population, especially in areas with a high prevalence of iron deficiency, should be covered by iron supplementation (a preventive approach). They indicated that in pregnant women whose caloric intake is more than 25% from animal sources and who have iron stores at the beginning of pregnancy, 30 mg daily is sufficient to maintain hemoglobin concentrations. However, under other circumstances 60 mg of iron daily, and up to 120-240 mg are recommended when animal sources provide less than 10% of calories, iron deficiency is prevalent and many women are anemic at the commencement of pregnancy. Importantly, they recommended that in areas where iron deficiency anemia is prevalent among school children, 30 mg of elemental iron, in the ferrous form, should be given daily to each child throughout the school year. Therefore, the distinction between therapy for anemia and prevention of iron deficiency was blurred and has remained so till now. The American Academy of Pediatrics has, since 1976, recommended iron supplementation for infants (4), but in contrast to antenatal iron suplementation which has become law in many countries, its implementation is not mandatory and in most countries is not practiced. Also, I know of no program that has implemented the recommendation of supplementing school children following the WHO guidelines, although there are school feeding programs that include vitamin and mineral enriched foods at levels intermediate between those generally accepted in fortification and supplementation programs.

The most recent document by INACG, WHO and UNICEF (5) continues, in part, this confusion, even in its title: "Guidelines for the use of iron supplements to prevent and treat iron deficiency anemia". In this document the highest oral iron dose to treat anemia has been reduced from 240 mg/d, or even higher doses, to 120 mg daily. Except for therapy, that includes a period of 3 months, the duration of preventive iron supplementation is not stated. Other WHO documents indicate giving several repeated courses of daily iron supplements to vulnerable populations (6). The rationale for this indication is lacking.

Although therapy and prevention are clearly different conceptually, the lack of definition in practice is understandable because the difference in correcting iron deficiency and anemia through therapy or through prophylaxis is a matter of the speed by which correction is indicated or desired. This basically depends on dose and duration. Obviously, in severe anemia, particularly when there is danger of decompensation or when permanent damage from anemia can ensue, therapy is important.

Present situation regarding iron deficiency and anemia and the effectiveness of current iron supplementation programs

The prevalence of iron deficiency and ferropenic anemia in the U.S. and in several industrialized countries has declined throughout the last 30 years but the contribution of iron supplementation to this decline remains unknown (7). Hallberg has suggested that self-prescribed iron supplementation, mostly through the intake of multivitamin-multimineral over the counter nutritional supplements has contributed significantly to the decline of ferropenic anemia observed in Sweden, but he has retracted earlier estimates of the relative contribution of this practice. I am sure that self-prescribed iron supplement intake does contribute, in the long term, to lowering the prevalence of iron deficiency and to increasing iron reserves.

The latest figures on prevalence of anemia in "non-industrialized countries" according to the WHO Micronutrient Deficiency Information System are presented in the following table (8):

Children				Women				Men	
0-4 y.o.		5-14 y.o.		All		Pregnant		15-59 y.o.	
%	(millions)	%	(millions)	%	(millions)	%	(millions)	%	(millions)
34	(190)	53	(526)	43	(535)	56	(54)	34	(456)

In industrialized countries the prevalence and the numbers of people with anemia are significantly lower, but there exist pockets of mostly poor populations where these prevalences are close to those in the non-industrialized world, particularly among infants and pregnant women. In California there are many counties where anemia in low income, 1 to 3 y.o. children reaches prevalences above 20% (9), and we have repeatedly found an iron deficiency prevalence of close to 20% and ferropenic anemia in 9% among childbearing age women students in Berkeley (10).

Based on these figures, the overall anemia prevalence in non-industrialized countries is estimated at 1,707 million people, of which 54 million are pregnant women (3.3% of all anemics). If 80% of anemia is due to iron deficiency or has an iron deficiency component (an accepted estimate) and for each of these ferropenic-anemic subjects there is another individual with iron deficiency without anemia, the total number of iron deficient people in this group of countries reaches 2,731 million. These numbers bring forth not only the huge problem of iron deficiency but also the fact that even if

all gestational iron deficiency were eliminated (an utopic situation) the total prevalence of iron deficiency and anemia would be reduced only by 3.3%. The International Conference on Nutrition (11) has set the goal to reduce by 1/3 the prevalence of gestational anemias by the end of this millennium. This goal is far from being reached, in spite of almost universal legislation in the developing countries establishing antenatal iron supplementation. These figures are not intended to diminish the importance of addressing the gestational anemia situation, nor its priority. They intend to show that more general approaches besides antenatal iron supplementation must be considered if the total iron deficiency problem is to be resolved.

Nutrition education, food fortification with iron, public health measures to diminish various types of infections, adequate perinatal practices, and the correction of other accompanying anemia-causing nutritional deficiencies are important steps in this regard (7), and are covered in this symposium. However, targeted, community based, preventive iron supplementation should not be discarded as another viable option. I contend that this strategy should be considered with a higher interest than that which is presently receiving. The reasons for this are the following:

1- Dietary improvement to a level where bioavailable iron meets the needs of vulnerable populations is far removed in time, particularly in a vast majority of the developing world population, even with important advances in biotechnological improvements of foods to increase iron bioavailability.
2- Food fortification can improve iron nutrition of populations (12), but its vehicles often do not reach the target populations in a significant proportion (e. g. infants of low income families, rural subsistence farmers in the developing world). Important advances are taking place in this regard, as will be addressed in this Symposium. Our findings and those of others in the industrial world indicate that iron deficiency affects a significant population even with current practices of food fortification.
3- New developments in this strategy are taking place which could make targeted, community based, preventive iron supplementation affordable, safe, multinutrient, and with high coverage (please see below).

An analysis of the effectiveness of antenatal iron supplementation programs in the developing world is revealing and must be considered in the promotion of efficacious preventive iron supplementation. Several studies (13-17) demonstrate that current practices of antenatal iron supplementation in most countries of the developing world have failed and explore the causes of this failure. These can be summarized in problems on the supply side as well as on the demand or recipient side.

There is consensus that the dominant causes of failure are in the supply side, including the structure of the programs: a) poorly trained and/or motivated personnel at all decision and delivery points, these being essentially hospitals and health centers; b) inadequate coverage because a large proportion of pregnant women have little or no access to health centers (WHO estimates that 47% of the rural population in the developing world lack access to adequate health care) (18); c) insufficient and inconstant supply of the desired amounts of supplements; d) presentation and shelf life of iron supplements, and e) cost.

On the demand or recipient side, the causes of failure are mainly: a) poor knowledge of the importance of adequate iron nutrition and anemia prevention, leading to late consultation and poor adherence to the supplementation regimen; b) undesirable side effects secondary to the ingestion of iron tablets; c) lack of family and/or community support, and d) cost where free iron tablets are not supplied.

Considering coverage and adherence to antenatal supplementation programs, estimates of effectiveness of such programs in Latin America, including both urban and rural areas, do not reach 20% of intended (17). There are no figures on which to estimate the coverage and adherence of supplementation programs aimed at the infant and school children populations, but they are perceived as very low to nil.

New developments in the strategy of preventive iron supplementation

Given the synthesis of the problem of iron deficiency, the directives provided by WHO and other international agencies and specialized groups (e. g. INACG) and the poor effectiveness of currently operating iron supplementation programs in the developing world, we decided to explore new schemes of this strategy with emphasis on prevention.

Current recommended schemes are based on the daily administration of supplements either during pregnancy or in repeated cycles of 2-4 months or several weeks every year to population groups vulnerable to iron deficiency. The only widespread operating system in the developing world is almost exclusively directed to pregnant women and is fundamentally based in hospital and health center settings, with little active community participation. In many instances iron supplementation is directed towards the therapy of anemia (therapeutically-oriented supplementation) and is administered in high doses (often associated with undesirable side effects) only to those individuals already presenting anemia. An interesting development in this regard, is the pharmaceutical production of a gastric delivery system that improves iron absorption and reduces undesirable side effects (19,20). Unfortunately the commercial production of this preparation was never reached.

Based on a series of studies in animal models, and in humans (clinical studies and field trials), where efficacy, efficiency and safety of supplementation schemes have been evaluated, we are proposing the community-based, preventive, weekly iron + folate supplementation targeted to vulnerable

population groups. As indicated above, this strategy should be integrated with other measures aimed at controlling iron deficiency (and ideally at controlling other nutritional deficiencies), including the therapy of severe ferropenic anemia.

The studies we have performed are summarized as follows:

1- Efficacy and efficiency of intermittent iron supplementation:
a) Animal model. We developed an animal model where weanling rats were trained to meal-feed twice daily and to consume a small pre-meal containing the desired amount of iron. That way we could study iron-deficient and iron-normal rats, supplemented or not. We decided to study the effect of iron supplements administered to both iron-deficient and iron-normal at a dose comparable to that universally recommended to pregnant women where iron deficiency is prevalent and when they begin supplementation late in pregnancy. This dose is roughly 10 times the usual iron intake by unsupplemented women. Our findings (21) showed that iron absorption declined very rapidly when iron was administered daily while it declined at a slower rate when the dose was administered every 3 days (in synchrony with gut mucosal turnover). Iron deficiency, measured by blood hemoglobin and liver iron levels were restored equally fast with both regimens, suggesting that the efficacy in repleting the iron deficit was similar. Calculations of iron absorption rates, adjusted to the same level of total iron absorption prior to the dose where radioactive whole-body counting measurements were made, indicated more than a 2 fold higher efficiency in the every-3 days iron deficient supplemented animals. We recognize the limitations of this animal model and the risks incurred in extrapolating these results to the human.
b) Human iron absorption studies. The pioneering work of Dr. Layrisse and collaborators in this regard must be recognized and admired, and specifically pertinent to this presentation is his contribution in measuring the absorption of therapeutic doses of iron under different conditions (22). Importantly, they showed that iron deficient subjects could absorb as much as 22% of a 60 mg dose of iron as FeSO4 when consumed away from meals, that when consumed with meals, or immediately before or after meals, absorption decreased to less than half, and that the addition of ascorbic acid to that "therapeutic" iron dose had a small effect but in an iron deficient-anemic case absorption reached 29.5 %.
The question of whether weekly iron supplementation in the human is more efficient than daily supplementation, has provoked several studies which can be summarized as follows:

i) In normal or mildly iron deficient populations, mean iron absorption from a dose of 50 - 60 mg of iron as FeSO4 is between 8 and 10%, in the absence of meals (23) showed, in Kansas, that iron absorption was only 13% lower when measured after one week of daily supplementation with 50 mg of iron when compared with the absorption after not receiving iron supplements for one week. If an outlier is removed, absorption is 30% lower. Importantly, absorptions as high as 28% were observed in iron deficient subjects when iron was administered without a meal. They demonstrated also a dramatic reduction in percent absorption when the supplements were ingested with food. Pizarro et al. (unpublished, cited in 24) using an identical design to that of Cook and Reddy (23) apparently reached similar conclusions in Chile. In a study of longer duration, Viteri et al (25) also found in a population in California, about a 13% decline in iron absorption from a 60 mg dose after one week of daily supplementation. Maximal absorptions were also between 25 and 30%. However, after two weeks of daily iron supplementation, mean iron absorption had declined to 6% and maximal iron absorption was only 12%. In contrast, iron absorption in subjects receiving 60 mg of iron weekly remained elevated after two weeks (3 doses) and even after 4 weeks iron absorptions of 21 and 20% were still observed. When adjusted to a plasma ferritin of 20 µg/L, mean iron absorption from weekly doses was stable at 14-15% even after 4 weeks of supplement intake (5 doses), while mean absorption in daily supplemented subjects was reduced to 7%. Adjusted percent iron absorption from weekly 120 mg doses, although slightly lower than those from 60 mg doses, do not differ significantly. Therefore, the total iron absorbed from 120 mg weekly doses is close to double that absorbed from 60 mg doses.

ii) In iron deficient, anemic subjects in Dakar, Senegal, results are essentially similar but at higher percent absorptions. In the case of weekly supplementation, maximal absorptions remained above 35% for 2 weeks and from then on they declined to 24 and 20% after 5 weekly doses. By then subjects receiving daily iron were absorbing only 10% of the dose. Weekly supplemental iron absorption adjusted to a plasma ferritin of 20 µg/L again showed a greater stability than daily iron absorption and did not differ from the values obtained in the Berkeley subjects. Adjusted daily iron absorption in Senegal remained higher than in Berkeley but showed a similar 30% decline between the first and the last two week periods of study. Again, adjusted percent iron absorption from weekly 120 mg doses, although only 70% of that from 60 mg doses, does not differ significantly. Therefore, the total iron absorbed from 120 mg weekly doses is substantially higher than that absorbed from 60 mg doses.
These results reinforce the following: a) Even when daily iron absorption declines more rapidly than weekly, total mg of iron absorbed during the month of study is

higher than that absorbed from weekly supplementation, and therefore, daily iron supplementation is recommended for therapy. And b) Total iron absorbed from weekly 60 mg supplements in iron deficient subjects can reach over 20 mg of iron per week, or the equivalent of 3.2 mg of extra iron daily. With 120 mg/ week, iron absorption can reach the equivalent of 5.4 mg of extra iron/day.

c) Field trials. The following table, summarizes the information available to me and to the Task Force for the control of iron deficiency of the ACC/SCN actualized to early 1999.

Weekly iron supplementation efficacy trials

Country status	No OF Studies	Population	No OF Subjects	Results
Brazil	1	6 - 12 m, children	1,280 Ua	+wb Prepc.
France	1	6 - 36 m, children	204 U	+ w Sub
Indonesia	1	6 - 36 m. children	442 S	+ d and w
			390 U	+ d; w ± Draft
China	1	6 - 36 m. children	246 S	+ d and w; - p Publ.
China	3	3 - 6 yr old children	673 S	+ d and w Publ.
Indonesia	2	3 - 6 yr old children	65 S	+ d and w Publ.
			289 U	+ w Prep.
Viet Nam	1	1 - 2 year old children	300 S	+ d and w Publ..
Bolivia	1	3 - 6 yr old children	300 S	+ d and w Publ.
Indonesia	1	School-age children	545 S	+ d and w Publ.
Guatemala	1	School-age girls	350 S	+ d and w Publ.
Panama	2	School-age children	5,021 S	+ d and w Prep.
Mali	1	School-age children	545 S	+ d and w Draft.
Indonesia	1	Adolescent girls	273 S	+ d and w Publ.
Malaysia	1	Adolescent girls	590 S	+ w Publ.
Tanzania	1	Adolescent girls	237 U	+ w plus motiv. Prep.
Peru	1	Adolescent girls	296 U	+ d; ± w; - p Prep.
United States	1	Fertile-age women	116 U	+d and w In press.
Indonesia	2	Fertile-age women	380 S	+ d and w Publ
Indonesia	2	Pregnant women	139 U	+ d and w Publ.
		Pregnant women	?? S	+ d; ± wc; - p Publ.
China	1	Pregnant women	389 S	+ d and w; - p In Press
Guatemala	1	Pregnant women	218 U	+ d and ± w Draft.
Malawi	1	Pregnant women	413 S	+ d and w Prep.
Venezuela	1	Pregnant women	104 U	+ bi-w; + w Prep.
Mexico	1	Pregnant women	165 U	+ dc and ±wc Prep.

TOTAL 16 Countries 30 studies through the life cycle except the elderly, involving 13,900 + subjects. 13 published; 2 in press; 1 submitted; 3 in draft form, and 8 in preparation. Results were positive for daily and weekly supplementation in all studies where the supplement was administered under supervision. In 4 of 9 unsupervised, unmotivated studies weekly is ±.

a U = unsupervised intake; S = supervised intake. One study comparing weekly and twice weekly iron supplementation in pregnant women. Slight advantage of twice weekly dosing.

b + results are better than placebo and/or are comparable to daily supplementation, considered the «golden standard» in terms of the efficacy. ± = less efficacious than daily or of doubtful efficacy. In the case of pregnancy, however, ± means no significant improvement in Hb or ferritin between early pregnancy and term. It must be recognized that in the absence of iron both fall. Therefore, ± in pregnancy is positive in preventing the otherwise expected deterioration. - = deterioration. w = weekly; d = daily; p = placebo.

c Prep. = in preparation; Draft = in draft form; Sub. = submitted; Publ. = Published.

These results demonstrate the efficacy of weekly iron supplementation in supervised field trials, where overall results in terms of hemoglobin and ferritin levels are no different from those of daily iron supplementation, except in pregnancy and in some unsupervised trials where daily iron supplementation is often more efficacious and effective respectively. In spite of these differences, which are small and most often neither statistically or biologically

significant, weekly iron supplementation improves iron nutrition significantly when compared to current practices. In the specific case of pregnancy, weekly iron supplementation has proven effective in preventing hemoglobin levels below 90 g/l at term. Risk of perinatal complications increase when hemoglobin concentrations fall below this level (26). An important factor in the success of antenatal iron supplementation is the level of iron stores and hematological condition with which women enter pregnancy (27). Several studies have demonstrated that the most important determinant of hemoglobin level at term is the hemoglobin level early in pregnancy, even among women receiving supplements (28). This should serve as a strong incentive to promote "pre-pregnancy iron health", including iron reserves in the order of 300 or more mg (29-32). Preventive weekly supplementation with iron and folate to women prone to become pregnant cannot only achieve the desired iron health but also a safe level of folate nutrition that will prevent neural tube defects. We have recently demonstrated in a group of non-pregnant Berkeley women that weekly folate at a dose of 3.5 mg sustains plasma and red cell folate at desirable levels. Currently we are determining if 2.5 mg a week will achieve similar levels.

2- Studies on the safety of different iron supplementation schemes.

Iron excess is dangerous because it promotes the development of reactive oxygen species (ROS) which in turn produce oxidative damage to proteins, nucleic acids and lipids, altering cell membrane and cell duplication and function secondary to altered enzymatic functions (33, 34). Iron excess can be chronic as in the case of genetic hemochromatosis or temporarily produced by the ingestion of iron at doses that surpass the antioxidant capacity of the body.

The results from our animal studies which demonstrated that daily iron supplementation at 10 times the normal level of iron intake (comparable to ingesting 120 mg of supplementary iron daily in the adult human) maintained a high level of intestinal mucosal and liver iron, prompted us to explore if this situation resulted in oxidative stress secondary to the production of ROS. In order to measure this condition we developed a method to measure ethane and pentane exhalation rates (35,36), and in collaboration with Drs. B. N. Ames and P. Walter, we modified a specific method to measure malondialdehyde in the presence of high levels of tissue iron (37) and have measured mitochondrial DNA damage and respiratory function in liver and kidney. Ethane and pentane are end products of lipid peroxidation and malondialdehyde (MDA) is a byproduct of lipid hydroperoxides. Of these three biomarkers of oxidative stress, ethane is the only that is not metabolized to any significant extent in the body, making it the most sensitive indicator of what we call early iron overload conditions (EIOC). However, in the presence of excess iron, ethane can be produced by intraluminal processes in the gut, making it less specific of tissue peroxidative damage. On the other hand, elevations in tissue and plasma MDA concentrations are very specific indicators of tissue oxidative stress. At the same time, we adapted and validated the breath ethane and pentane methods to measure oxidative stress in humans. A summary of recent results follow:

i) In daily supplemented, as well as in iron deficient rats, elevated liver and kidney MDA and ethane exhalation rates, impaired mitochondrial respiration and significant mitochondrial DNA ruptures are present and are strongly correlated with the excess liver iron observed in daily supplemented rats, suggesting a situation of EIOC (38,39).

ii) Every 3-day iron supplementation significantly ameliorates these indicators of EIOC in rats. They are present the day after the iron dose but by the third day they are back to normal levels. Altered mitochondrial respiration and DNA damage are not observed (38, 39).

iii) In non-pregnant women supplemented with 120 and 98.5 mg of iron for 4 and 6 weeks, respectively, ethane exhalation rates and plasma MDA levels are also elevated, as are % saturation of transferrin and plasma ferritin levels (40,41). In weekly supplemented women with 120 mg of iron, ethane exhalation rates are elevated the day after the ingestion of the dose but by the second day most are back to non-supplementation levels. Plasma MDA is also elevated the day after the iron dose, but by the seventh day is back to pre supplementation levels, as are the % saturation of TIBC levels. Serum ferritin is not elevated in these women (40).

iv) In field trials, a significant number of women and children supplemented daily exhibit serum ferritin levels that are beyond the levels corresponding to the 80th percentile of normal populations. This phenomenon is not observed in the same population groups receiving weekly iron supplements and where serum ferritin distributions have been analyzed, they are superimposable to those observed in normal populations (42).

v) Several studies, but not all, demonstrate that undesirable side effects are higher in daily supplemented groups, that adherence to the supplementation regimen is lower among those with side effects and that rejection of the regimen is higher among those receiving daily iron (43,44).

CONCLUSIONS

Iron supplementation, should be conceived as a targeted, long-term, safe preventive measure that must be differentiated from iron therapy. In the long-term it should correct mild-moderate iron deficiency and ferropenic anemia and should increase iron reserves without producing early iron overload.

Preventive iron supplementation should be conceived as another strategy for the control of iron deficiency and should be complementary to other interventions aimed at controlling iron deficiency, including iron therapy when this is indicated.

Daily iron supplements at doses of 120 mg/d, consumed away from meals, is absorbed by iron deficient subjects at levels which are therapeutic in the short term, even for pregnant women. Weekly iron supplementation at doses of 60 and 120 mg/d can provide enough iron to correct iron deficiency in a medium term and to prevent and correct mild-moderate iron deficiency even in pregnant women, if administered from early pregnancy.

Field trials demonstrate that long term weekly iron supplementation is efficacious in improving iron nutrition of vulnerable groups.

Animal studies demonstrate that daily iron supplementation produces constant oxidative stress while this stress is less and of short duration when iron is administered intermittently. Studies in humans reach the same conclusion.

Extended population-based studies should be undertaken to determine if well designed community-based programs are sustainable and effective.

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Iron fortification with special reference to the role of iron EDTA

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SUMMARY. Iron fortification has been used for decades in a number of industrialized countries to combat iron deficiency and seems to have played a significant role in reducing its prevalence, especially in infants and women. The overall strategy has been one in which staples such as wheat, flour, have been fortified with iron. While the effects appear to have been positive, there are still problems not yet completely resolved. In this context, the selection of the fortificant always represents a compromise between a choice of chemically reactive compounds of high bioavailability, such as ferrous sulfate, and inert compounds, which are poorly absorbed. Ferrous sulfate is very effective when added during the preparation of bread and bakery products and infant formulas, but cannot be used in stores flour because of organoleptic problems and inert compounds, such as elemental iron powders, have to be used. The search, therefore, continues for compounds of high bioavailability which do not cause organoleptic changes in the vehicles to which they are added. Problems associated with effective iron fortification programmes are compounded in a number of developing countries by a variety of factors. Most potential vehicles are not centrally processed, inhibitory ligands in staple cereal diets depress the absorption of both intrinsic and fortification iron, anemia is often of multifactorial in etiology, financial resources are scanty and governmental support sometimes lacking. Despite such difficulties there are encouraging signs of progress in a number of countries, using a variety of fortificants and vehicles. In the present review particular attention is paid to the potential role of NaFeEDTA as a fortificant in developing countries. It is much less affected by the inhibitors of iron absorption present in diets of low bioavailability, it can be added to a number of vehicles without causing organoleptic problems and its efficacy has been underlined in three intervention studies.

Keywords: Iron deficiency, iron fortification, iron EDTA, NaFe EDTA, iron bioavailability.

RESUMEN. La fortificación de alimentos con hierro: papel del hierro EDTA. La fortificación con hierro ha sido ampliamente utilizada por varías décadas en muchos países industrializados para combatir la deficiencia de hierro y parece haber jugado un papel significatiivo en su reducción, particularmente en niños y mujeres. La estrategia general ha sido la fortificación con hierro de alimentos básicos como la harina de trigo. Aunque los efectos parecen ser positivos, aún persisten problemas por resolver. En este contexto, la selección del fortificante siempre representa un compromiso entre compuestos químicamente reactivos de alta biodisponibilidad como el sulfato ferroso y los compuestos inertes, de muy poca absorción. El sulfato ferroso es muy efectivo cuando es agregado durante la preparación del pan y productos de panificación y de fórmulas infantiles, pero no puede usarse en harinas de reserva por los problemas organolépticos, por lo que compuestos inertes de hierro elemental en polvo tienen que usarse. La búsqueda por compuestos de alta biodisponibilidad que no causen cambios organolépticos en los vehículos al que son agregados continua. Los problemas asociados a la efectividad de los programas de fortificación en países en desarrollo están influenciados por una variedad de factores. La mayoría de los vehículos potenciales no son procesados centralmente, existen ligandos inhibidores en las dietas básicas que deprimen la absorción tanto del hierro intrínseco como del hierro agregado. la anemia es de etiología multifactorial, los recursos financieros son limitados y el apoyo gubernamental está a veces ausente. A pesar de todas las dificultades, hay signos de progreso prometedores en varios países, utilizando una variedad de fortificantes y vehículos. En la presente revisión se ha dado particular atención al papel potencial del hierro EDTA como fortificante en países en vías de desarrollo. Este compuesto está menos afectado por los inhibidores de la absorción de hierro presente en las dietas de baja biodisponibilidad, puede agregarse a varios vehículos sin provocar cambios organolepticos y su eficacia ha sido afirmada en tres estudios de intervención.

Palabras clave: Fortificación, hierro EDTA, biodisponibilidad, deficiencia de hierro, anemia.

INTRODUCTION

There has been a steady decline in the prevalence of iron deficiency anemia in industrialized countries over the last several decades, with the drop being particularly striking in two most vulnerable groups, infants (1) and women (2,3). The prevalence in Sweden dropped from between 25 and 30% in the 1960's to about 7% in the mid seventies (2), while an even lower figure of 2.6% was noted in the USA in the NHANES II survey carried out between 1976 and 1980 (3). The low prevalence in the USA was confirmed in phase 1 of the NHANES III survey (1988-1991), with only 3 to 4% having iron deficiency anemia (4). The improvement in iron nutrition, both in infants and women, has been ascribed, in part at least, to the high consumption of iron-fortified foods. It is not, however, the only factor. Over the same period, there has been

a significant decrease in menstrual blood losses due to increased use of oral contraceptives and 'over the counter' preparations containing iron and vitamins have been widely consumed (2). In addition, there are also other factors which stem from increased affluence, including smaller families and a wider selection of foods (2).

In contrast to affluent industrialized countries, iron deficiency anemia remains a major and pressing problem in most of the developing world (5). This can be ascribed to a number of factors. The major one is the low bioavailability of the iron in staple diets, composed largely of grains and legumes (6). The problem is compounded in many areas by intestinal worm infections and particularly blood loss from hookworm infestation (7). In addition, associated conditions, such as malaria, HIV infection and vitamin A deficiency, might be expected to blunt the effects of any intervention programme (8-11) whether it involves fortification (12), supplementation (13) or dietary modification (14).

It is against this background that the status of iron fortification in the world today must be viewed. On the one hand, there is the example of industrialized countries, such as the USA, in which a number of foods have been successfully fortified, while on the other, there is most of the developing world in which it has proved extremely difficult to develop viable programs to prevent iron deficiency through food fortification. In the present review, factors affecting the availability of fortification iron will first be discussed. The current status of iron fortification in developed countries will then be covered briefly, while major attention will be directed to the constraints that have limited the successful application of similar approaches in most developing countries. In this context, it should be noted that the relative advantages of different iron fortificants and vehicles have been addressed in some detail over the last years in several reviews (12,15-25). In seeking ways of overcoming current challenges, particular attention will be paid to the potential value of NaFeEDTA as a fortificant, since it has been shown to be effective in three pilot trials carried out in developing countries (26-29). It is perhaps appropriate that this compound should be discussed at a meeting in honor of Doctor Miguel Layrisse, since his own group was involved in seminal studies demonstrating its beneficial effects on the absorption of iron from food (30).

FACTORS AFFECTING BIOAVAILABILITY OF IRON FORTIFICANTS

The amounts of fortification iron absorbed from a particular diet are dependent on three factors. These include the composition of the diet, the iron status of the individuals consuming the diet, and the relative bioavailability of the iron fortificant (22). There are two types of iron in the diet, heme iron (derived from hemoglobin and myoglobin) and nonheme iron (derived mainly from cereals, vegetables, and fruit). Most forms of nonheme iron in a meal, whatever their origin, enter a common pool during digestion and are thus equally susceptible to a number of promoters and inhibitors of iron absorption (31). The major promoters of iron absorption are meat and ascorbic acid (31) while the major inhibitors are phytates (32) and polyphenols (33).

Soluble iron fortificants, such as ferrous sulfate, enter the common pool of nonheme iron completely and are absorbed to the same degree as is the intrinsic nonheme iron in the diet. Such iron is thus well absorbed when the diet contains adequate amounts of ascorbic acid and/or meat, while it is poorly absorbed from diets in which inhibitors of iron absorption predominate (16). Ferrous sulfate and other soluble iron complexes can only be used as fortificants in certain limited situations, since they are chemically reactive and tend to produce undesirable organoleptic changes in the vehicles to which they are added (15,23,25). As a result, several other fortificants, which are less soluble under the conditions prevailing in the upper gastrointestinal tract, are in common use. Although such inert compounds do not cause organoleptic changes when stored in a variety of vehicles, they tend to be less well absorbed.

A list of currently available fortificants, including the vehicles to which they can be added, their relative bioavailabilities in comparison with ferrous sulfate $7H_2O$. (designated as 100), their iron contents and relative costs are shown in Table 1, which is adapted from previous ones by Hurrell (12,19,23,25) and Bothwell and MacPhail (22). Their relative bioavailability has been assessed on the basis of their physical characteristics (particle size, relative surface areas and solubility in acid), their capacity as compared with ferrous sulfate to restore the hemoglobin level in iron deficient rats and the degree to which they exchange with the common pool of nonheme iron in the diet in human absorption studies using radioactive or stable isotopes (23,25). While reasonable agreement between the various methods has been reported (34), there are still doubts about results obtained when testing compounds, such as elemental iron powders, which are poorly soluble in dilute acids. In such circumstances there are always questions as to whether the labelled experimental compound has the same physicochemical characteristics as the commercial compound (12). This may explain the wide range of values for relative bioavailability found in different human experiments (e.g. 13 to 148 with reduced iron).

Included in Table 1 is the interesting compound, ferrous bis-glycine, which is currently being actively studied. This water-soluble complex is not only highly bioavailable but can also be added to a number of foodstuffs without causing organoleptic changes (35). The degree, if any, to which it is protected from inhibitory ligands in the diet, is a topic of active current debate. In one study in adult human subjects the chelated iron was absorbed several fold better than ferrous sulfate when fed in a maize meal (36), while in another, it was no better absorbed than ferrous sulfate when given to infants in a vegetable weaning food (37).

TABLE 1
Iron sources and vehicles used in food fortification
(Adapted from Hurrell (12,19,23) and Bothwell and MacPhail (22))

	Approximate Fe content (%)	Vehicles in use[a]	Average relative bioavailability[b] Rat	Man	Approximate relative cost[c]
Freely water soluble					
Ferrous sulfate $7H_2O$	20	1,3,7[d]	100	100	1.0
Dried ferrous sulfate	33	1,3	100	100	0.7
Ferrous gluconate	12	1,3	97	89	5.1
Ferrous lactate	19		-	106	4.1
Ferric ammonium citrate	18	1,3	107	-	2.1
Ferrous bis-glycine	20	1,2,3	116	90-400[e]	6.0
Poorly water soluble/soluble in dilute acid					
Ferrous fumarate	33	2,5	95	100	1.3
Ferrous succinate	35	2,5	119	92	4.1
Ferrous saccharate	10	2,5	92	74	5.2
Water insoluble/poorly soluble in dilute acid					
Ferric orthophosphate	28	2,7	6-46	25-32	4.1
Ferric ammonium orthophosphate	19	4	-	30-60	-
Ferric pyrophosphate	25	2	45-58	21-74	2.3
Elemental Fe powders: electrolytic	98	2,4	44-48	5-100	0.5
carbonyl	98	2,4	39-66	5-20	1.0
reduced	97	2,4	24-54	13-148	0.2
Protected compounds					
NaFeEDTA	14	6,8	-	100-290	6.0
Hemoglobin	0.34	2,4	-	100-700	-

a 1 : infant formulas; 2 : infant cereals; 3 bakery products; 4 : flour; 5 : beverages; 6 : sugar; 7 : salt; 8 : condiments
b The relative bioavailability of individual fortificants is expressed in relation to ferrous sulfate 7H20, which is designated as having a relative bioavailability.
c The approximate costs relative to ferrous sulfate $7H_2O$ for the same amount of total iron. None of the figures of relative costs takes into account the relative bioavailability of the different compounds.
d Ferrous sulfate, together with sodium acid sulfate and orthophosphoric acid, has been used to fortify salt in India. (58)
e Based on only two studies. Results in the one suggest that ferrous bis-glycine may be a protected compound. (36)

CURRENT IRON FORTIFICATION POLICIES IN INDUSTRIALIZED COUNTRIES

From a theoretical standpoint iron fortification is the best long-term approach for combating iron deficiency. It can reach all segments of the population, does not depend on individual compliance and is cheap. In addition, depending on the vehicles chosen for fortification, it can cover the whole population or be targeted at particular vulnerable groups, such as infants and children. There are, however, still certain unresolved technical problems and theoretical questions relating to the application of fortification strategies which merit discussion. These relate to the efficacy of some of the compounds currently in use and to the potential hazards of universal fortification.

Fortification of infant formulas and cereals

The two major sources of fortification iron in infancy are infant formulas and infant cereals. Milk- and soy-based formulas tolerate well the addition of highly bioavailable soluble salts, such as ferrous sulfate, and the use of fortified formulas (± 12 mg iron per reconstituted liter) increased in the United States from 40% to 80% between 1971 and 1985 (38). Indeed, their use has been identified as an important factor in the declining prevalence of anemia, both in low-income and middle-class infants in the United States (1). As a result, the US Committee of Nutrition has recommended that all infant formulas be fortified (39). The efficacy of iron-supplemented milk-based formulas has been further supported by the results of trials in infants (40). It should be noted that the bioavailability

of iron is considerably enhanced by ascorbic acid (41-44). The weight ratio of ascorbic acid to iron should be at least 5 to 1 in such formulas (41,44). Using this approach, the bioavailability of fortified formulas is so high that it has recently been suggested that it may be appropriate to reduce the current level of iron fortification of ± 12 mg iron, since it allows for the absorption of approximately twice the infant's requirements (45).

Infant cereals are more difficult to fortify with iron. Not only do they have a high phytate content but they are also very sensitive to fat oxidation, so that highly bioavailable forms of iron, such as ferrous sulfate, cause organoleptic problems (12), while more stable forms of iron are much less bioavailable (46). Such preparations can, however, still be effective, even when the fortificant is electrolytic iron, provided very large amounts are present (55 mg iron/100 g dry cereal) (47). Alternatively, ferrous fumarate, which is poorly soluble in water but soluble in acid, can be used as the fortificant (48). As with infant formulas, absorption from infant cereals is enhanced by the presence of adequate amounts of ascorbic acid (48).

While iron deficiency is a less critical problem in childhood than it is in late infancy, school programs lend themselves to targeted iron fortification. In this context, milk has proved an effective vehicle (49) and milk-based chocolate drinks fortified with ferrous succinate and ascorbic acid, can be usefully targeted to children and adolescents (50).

Fortification of wheat and other cereal products

Bakery products and wheat flour are currently the most frequently used vehicles for iron fortification that reach the whole population, with wheat flour enrichment mandatory in the USA, United Kingdom and Denmark but not in France, Italy and Spain (25). In those countries in which iron fortification of flour is mandatory, the actual extent of fortification varies widely - up to 44 mg/kg in the USA, 30 mg/kg in Denmark and only 16.5 mg/kg in the United Kingdom (25). In the USA it has been calculated that the iron contribution from foods that are normally fortified, such as whole bread, rolls, crackers, corn flour, corn grits, pasta and breakfast cereals, represents more than 20 percent of the dietary intake (25).

Insofar as cereal products are concerned, iron has been added for the most part in two forms, as ferrous sulfate and as elemental iron powders. Ferrous sulfate is very satisfactory as a fortificant when added during the preparation of bread and bakery products, since it is absorbed as well as the intrinsic iron in the wheat. It is also widely used to fortify infant formulas, pasta and cereal flour, which are only stored for short periods. The more widespread application of ferrous sulfate and other water soluble ferrous salts is limited by their chemical reactivity, which causes organoleptic problems. As a result, several other compounds which are poorly soluble in dilute acids, including gastric juice, have been the most widely used compounds in the fortification of cereals. They include elemental iron powders and ferric ortho-and pyrophosphate. While hemoglobin regeneration studies in rats suggest a relative bioavailability about half that of ferrous sulfate, studies in humans have given conflicting and variable results (22). As previously discussed, this could be due to the fact that the compounds tested had different physicochemical characteristics from those produced commercially and to the influence of meals on their dissolution in gastric juice (34,51). Such variable results have made it difficult to assess the overall effects of flour fortification on iron nutrition.

The current dilemma is underlined by the Swedish experience. Until 1995 wheat flour was heavily fortified with carbonyl iron (65 ppm), the elemental powder with the smallest particle size and the highest solubility in gastric acid (21). It was calculated that it provided up to 40% of the dietary iron intake in Sweden and there seemed good reason to believe that it had played a significant role in reducing the prevalence of iron deficiency in the country (2). When, however, the relative bioavailability of radioactive carbonyl iron was measured in human subjects it was very low, varying between 5 and 20% depending on the nature of the meal (52).

The doubts raised by such findings may be resolved over the next few years, since the fortification of flour in Sweden was stopped in 1995. Monitoring the iron status of the population, and especially premenopausal females, should therefore provide a unique opportunity of finally deciding what the impact of Sweden's national program of iron fortification actually was. While no firm data are yet available, there is already some indirect evidence that iron fortification may, indeed, have been exerting an effect. It has recently been shown that subjects with the iron-loading disorder, hereditary hemochromatosis, who have had their increased stores removed by repeated venesections, now reaccumulate iron more slowly (53). Calculations based on their need for maintenance phlebotomies before and after the cessation of iron fortification, suggest that an average of 0.65 mg fortification iron was previously absorbed by these subjects daily (53).

Safety of universal iron fortification

While there is still debate on the relative efficacy of universal fortification programes, this debate is much less vigorous than the one centering on the safety of such programmes. It has been argued that attempts to reduce the prevalence of iron deficiency in women of fertile age expose iron replete subjects to excessive quantities of iron. In this context, it should be noted that iron deficiency anemia was noted in only 0.2% of adult men in the NHANES II study in the USA (3). At particular potential risk are subjects homozygous for the HFE mutation, which is responsible for the iron - loading disorder, hereditary hemochromatosis (54). It occurs with a prevalence of about 0.3% in Caucasoid populations (55). From what is know of iron balance it seems likely that increasing the quantities of bioavailable iron in the diet, would have two effects. Firstly, subjects destined to present clinically.

iron overload due to the pathologic effects of excessive iron deposits would do so at younger ages than they would do otherwise. Secondly, iron fortification, would be expected to cause a proportion of asymptomatic homozygotes with only moderate iron overload to accumulate enough iron to develop clinical symptoms. Recently the debate has widened, with disturbing claims, based on epidemiologic data, that normal subjects with only modestly raised iron stores are at greater risk of developing ischaemic heart disease (56). While such claims have not gone unchallenged (57), the debate does raise issues relating to the desirability of fortification programs which supply increased amounts of iron not only to these who need it but also to those who do not.

As a result of these various doubts concerning universal fortification, increasing attention needs to be focussed on programs that are targeted at the most vulnerable sectors of the population. These include infants, young children and pregnant mothers. To do this effectively needs a multiprolonged approach. As mentioned in an earlier section, infant formulas and cereals can be effectively fortified with iron, with a resulting improvement in iron nutrition. In addition, fortified food items such as cookies and beverages, can be administered as part of school feeding programs (12). Insofar, as women of fertile age are concerned, targeted fortification is obviously not feasible and more effective supplementation programs may have to serve as an alternative. Programs are currently being advocated to extend supplementation beyond pregnancy to include adolescent and young adult females, with the emphasis on intermittent supplementation at schools, clinics and in the work place (13).

IRON FORTIFICATION IN DEVELOPING COUNTRIES

While several developing countries, including Chile, Guyana, Kenya, Zambia and Nigeria require that iron be added to flour (21), logistic problems have largely prevented the development of fortification programs in the majority. There are several reasons for this. Firstly, most potential vehicles are not centrally processed in a number of countries and, as a result, the use of alternative ones has been explored. These have included salt (58), sugar (27,29) and condiments (26,28). The second problem relates to the predominantly cereal diets consumed by many of the poorer populations. They are of low bioavailability insofar as iron is concerned. This means that any fortification iron that is added to such diets is equally poorly absorbed. An equally important constraint has been the low priority that the prevention of iron deficiency has had on the health agendas of many countries. Fortunately, there are signs that this is changing, with an increasing commitment to the development of coordinated programs for the prevention of micronutrient deficiencies, including iron deficiency (59).

Two major fortification strategies have been used in attempts to prevent iron deficiency in developing countries. The first involves the conventional approach of adding iron to staple cereals and the second is to administer the iron in forms that are less susceptible to inhibitory ligands in the diet. In this latter approach the iron has been given together with enhancers of iron absorption, such as ascorbic acid (41) or sodium hydrogen sulfate (58), or in protected forms, such as hemoglobin (60,61) and NaFeEDTA (30,62,63). As discussed previously, ferrous bis-glycine, which has been shown to be effective in treating iron deficiency anemia, may also fall into this category (36).

Fortification of cereals and dairy products with iron

Fortification of cereals has not been widely applied in developing countries but programs are developing in several countries. Rice has been successfully fortified in the Philippines, using ferrous sulfate as the fortificant (64). It has been shown to be effective in a clinical trial and there are current plans for making iron-enriched rice more widely available (64). In addition, instant noodles, triply-fortified with encapsulated reduced iron, iodide and vitamin A, are being marketed in Thailand (65). Furthermore, milk and dairy products fortified with the chelate, ferrous bis-glycine, are currently available in a number of Latin American countries (35,66) as are a variety of Kellogg's cereal products fortified with NaFeEDTA. Of particular interest is a national program started in 1993 by the Venezuelan health authorities to fortify both precooked maize and wheat flour with ferrous fumarate and vitamins (67). The maize and wheat, which are enriched with 20 mg/kg and 50 mg/kg iron respectively, account for 45% of the total energy consumed daily by the lower economic sector. A preliminary survey after 1 year in groups of children suggested a significant drop in the prevalence of anemia and further follow-up studies should provide valuable data on the efficacy of the program (67).

Role of absorption enhancers in iron fortification

While there must be a number of other countries in which iron fortification of staple cereals could be applied, the nature of the diets consumed in such countries suggests that beneficial effects will be limited unless the effects of inhibitory ligands can be overcome. In this context, it is worth recalling pioneering Chilean studies which showed the beneficial effects on iron nutrition of infant formulae fortified with both iron and ascorbic acid (40). More recently the same group has shown that infant cereals fortified with high levels of elemental iron together with ascorbic acid could be beneficial in preventing iron deficiency (47).

Enhancers of iron absorption have also been used in studies in which salt has been the vehicle. In one study, a stable form of iron, namely iron orthophosphate, was used as a fortificant together with ascorbic acid (68). Refined salt tolerated the addition of this combination moderately well, but even more satisfactory results were obtained in India with a combination of iron orthophosphate and sodium hydrogen sulfate (69). When added to salt (1 mg iron/g salt), the color,

taste, storage properties and bioavailability were all reported to be good; furthermore, the fortified salt proved efficacious in a multicenter trial. Community trials of fortified salt are currently underway (58).

Fortification of cereals with dried bovine hemoglobin

An alternative approach to iron fortification is to use compounds for iron fortification that are themselves less affected by inhibitory ligands in the diet. The two compounds which have received most attention are dried hemoglobin and NaFeEDTA.

Hemoglobin iron is well absorbed because it is not released from the porphyrin ring prior to uptake by mucosal cells and is therefore not affected by the dietary inhibitors which reduce the absorption of nonheme iron (70). Stekel and his coworkers, who used hemoglobin to fortify cookies distributed as part of a school lunch program in Chile, found that the absorption of the heme iron was about 20%, which was equivalent to an absorption of 1 mg iron per day (60). In a further study by Stekel's group, a weaning food composed of extruded rice flour was fortified with 5% bovine hemoglobin (61). The geometric mean absorption of the iron in the hemoglobin was 14.2%, which is severalfold greater than figures previously obtained with various forms of inorganic iron (1). While problems in ensuring the sterility of hemoglobin during collection and storage could lead to problems in gaining approval for its use in some countries, its potential as a fortificant merits further investigation.

Potential role of NaFeEDTA as an iron fortificant

The peculiar advantages in food fortification of the iron chelate NaFe EDTA have been demonstrated in a number of physiologic and clinical studies, (26-30,71-74) and its potential use as a food additive has recently been reviewed in a monograph prepared by the International Nutritional Anemia Consultative Group (75). In this context, it should be noted that Na_2EDTA and Ca Na EDTA have been used by the food industry for a considerable time to protect foods from metal-induced organoleptic changes.

Chemistry

The hexadentate chelate EDTA (ethylene diamine tetraacetic acid) can combine with virtually every metal in the periodic table, with the nature of the metal complex formed depending on such factors as stability constants, molar ratios and pH (12). Binding to iron is favored in the acid environment of the stomach, but in the more alkaline surroundings of the duodenum the iron is exchanged in part for other metals, such as calcium, copper and zinc (75). EDTA acts, therefore, as a shuttle, protecting iron from inhibitory dietary ligands in the stomach, such as phytates and polyphenols, and releasing it in the duodenum, where it is absorbed (75). The absorption of iron from meals fortified with NaFeEDTA is controlled by the same physiologic mechanisms that determine food iron absorption, with the amounts of iron absorbed being inversely related to body iron stores (30,62,63,71). When NaFeEDTA is added to a meal, less than 5% of the EDTA is absorbed intact as metal complexes, with less than 1% being complexed with iron. Absorbed EDTA complexes are rapidly and completely excreted in the urine (63,72).

Effects on iron absorption

Since iron is less affected by inhibitors when given as NaFeEDTA, its relative bioavailability when fed with meals is between 1.05 and 2.8 times that of ferrous sulfate (17) (Table 2). Its relative bioavailability appears to be greater in meals with a high content of inhibitors (17). NaFeEDTA has another advantage as a fortificant. Within the lumen of the gut the iron dissociates from the EDTA and exchanges completely with the common pool of nonheme iron in the diet (63). As a result, when NaFeEDTA is added as a fortificant to the diet the relative bioavailability of the intrinsic iron is increased to the same degree as is the fortificant iron (30,62,63). It should be noted that a similar enhancement in iron absorption can be obtained if other complexes of EDTA, such as, Na_2EDTA, are administered. For example, when Na_2EDTA and ferrous sulfate were added in equimolar quantities to Egyptian Baladi bread, iron absorption was 5.3% as compared with 2.1% when ferrous sulfate was fed alone (73). The optimal ratio of EDTA to iron appears to be 1:2 (74).

Organoleptic considerations

NaFeEDTA causes many fewer organoleptic problems than most other water soluble compounds and is suitable for fortifying wheat flour, other cereal and legume products and many other foods (12,75). It has also been successfully added to condiments, such as fish sauce and curry powder, and to sugar (26-28). It does, however, cause the sugar to turn slightly yellow and when the sugar is added to tea it discolors it (12). It can, also, cause unwanted color changes when added to certain foods, such as chocolate drink powders and infant cereals containing banana and other fruits (12).

Intervention trials

Thus far, three fortification trials have been carried out using NaFeEDTA. The results in these three studies will be reviewed briefly:

NaEDTA-fortified fish sauce (10-15 mg/day) was provided to a Thai village for 1 year (26). Packed cell volume (PCV) values showed a significant rise as compared with a control village supplied with unfortified fish sauce. The largest mean change of + 4.6 was seen in a sub-group of women who were anemic at the start of the study. This change was calculated to be equivalent to an increase in body iron of about 190 mg, which represented an increase in iron absorption of about 0.5 mg daily (26,75).

TABLE 2
Comparison of the mean absorption of iron from meals fortified with $FeSO_4$ or NaFeEDTA in several different studies reviewed by Hallberg (17)

Number of subjects	Amount of iron added (mg)	Absorption (%) $FeSO_4$ (A)	Absorption (%) NaFe EDTA (B)	B/A	Reference dose absorption[a] (%)	Type of meal
7	2.5	3.5	9.8	2.80	76.8	Milk, rice, sugar formula
21	5.0	3.3	7.4	2.20	51.3	Black bean gruel, corn tortillas, wheat bread, coffee
12	3.0	7.7	18.0	2.30	49.3	Wheat dough
11	3.0	12.4	14.4	1.20	35.2	Sweet manioc
12	5.0	3.5	7.2	2.06	35.3	Maize porridge
18	5.0	3.9	6.3	1.62	43.0	Rice, boiled vegetables, curry
8	2.7	6.6	7.9	1.20	47.8	Wheat-oat meal
10	5.0	5.4	5.7	1.05	35.0	Hamburger, string beans, potatoes
11	3.0	1.6	4.1	2.58	30.8	Egyptian flat bread

a) The percentage absorption of a 3-mg dose of ferrous iron.
b) The results in the last study, (73) which were obtained by adding equimolar amounts of ferrous sulfate and Na_2 EDTA, were reported after Hallberg's review.

NaFeEDTA-fortified sugar (± 4.3 mg/day) was administered to 3 out of 4 Guatemalan communities for 32 months (27,29). All pregnant women and subjects with severe anemia received iron therapy or supplements and were excluded from the analysis. Interpretation of the findings was complicated by certain confounding factors, including differences in the initial iron status of the communities, distribution problems and variations in compliance. Despite, these drawbacks, iron stores in the fortified communities increased significantly, except for women aged 18 to 48 years, in one community and greater than 49 years in another. In addition, children in two of the communities showed a significant improvement in hemoglobin concentrations when compared with children in the control community.

NaEDTA-fortified masala (±7.7 mg/day) was administered for two years in an Indian community living in Durban, South Africa (28). While the prevalence of iron deficiency anemia in Indians was known to be high, they lived in an area where the local black population was iron replete, with a proportion suffering from dietary iron overload. In looking for a suitable food vehicle it was therefore important to identify a dietary component that was consumed by the target population but not by blacks. Curry powder or masala was found to have a number of advantages. It was universally consumed by the Indian population, most of it was obtained from one supplier and it tolerated well the addition of NaFeEDTA. It provided a further and unexpected dividend. Iron absorption from a typical meal was moderately enhanced in the presence of curry powder, an effect which was probably due to enhanced gastric acid secretion (76).

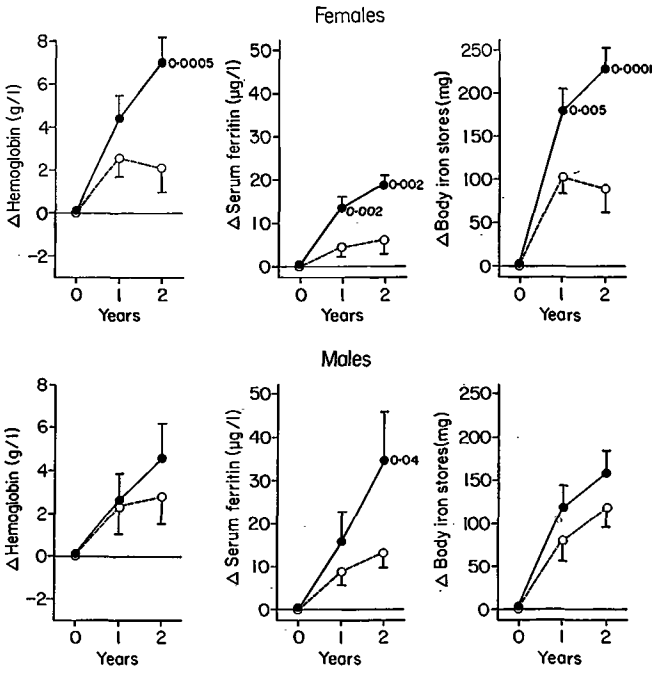
The trial differed from the other two studies in that it was double blinded and was conducted in a single community, with the 263 families randomly assigned to control and test groups, which were matched for iron status. Care was taken to ensure that crossover between groups did not occur and the masala, fortified or unfortified, was distributed directly to each family. In addition to evaluating the usual monitors of improving iron status (increasing hematocrit or hemoglobin and ferritin), an attempt was made to estimate the total body iron in each individual by using a composite of the hemoglobin concentration, percent transferrin saturation, and the serum ferritin concentration (3). This comprehensive index of iron nutrition made it possibly to compare subjects with wide variations in iron status and thus to assess both the beneficial and potentially adverse effects of additional iron, i.e.

development of iron overload (28).

A significant improvement in body iron as assessed by the index was detectable in the group of women receiving fortified masala after 1 year of the program (Figure 1). This improvement continued during the second year, when the rise in hemoglobin concentration became significantly greater than that in the control group. The prevalence of iron deficiency dropped dramatically in the women receiving fortified masala. Iron deficiency anemia was detected in 22% of individuals at the start of the study but only in 4.9% after 2 years of fortification. The most significant improvement in iron status was noted in women who entered the trial with iron deficiency and especially in those with anemia. Those with anemia showed an increase in calculated body iron of 505 mg, which is equivalent to the absorption of an additional 0.7 mg iron/day. The latter figure is close to the predicted improvement in iron balance of 0.8 mg/day based on radioisotope absorption studies using NaFeEDTA-fortified masala (76).

FIGURE 1

Changes (Δ) in measurements of iron status after 1 and 2 years of iron fortification in fortified (Ɔ) and control (O) groups of males and females (mean ± SE). The probability (one tailed, Student's test) that individual changes were greater in the fortified group than in the control group are also shown (Source: Ballot et al (28); with permission)



In iron replete males the rise in calculated body iron was modest and reached statistical significance only in alcohol abusers receiving fortified masala. This suggests that iron-replete males are unlikely to accumulate excessive amounts of iron under these fortification conditions.

Regulatory issues relating to the use of NaFeEDTA

With so much evidence suggesting that NaFeEDTA is a potentially effective food fortificant, it is important to examine the nature of the constraints that have limited its more widespread use. Based on animal toxicological studies, the Joint FAO/WHO Expert Committee on Food Additives (JECFA) sanctioned in 1974 the use of other salts of EDTA, ($CaNa_2EDTA$ and Na_2EDTA) as food additives up to 2.5 mg/kg body weight/day, with a maximal acceptable daily intake of 150 mg/person/day (77). They are useful as sequestering agents to prevent organoleptic changes in canned products and foods, such as mayonnaise, other sauces and margarine, and are used in many countries throughout the world. In the USA they may be added to 34 different foods and the estimated daily intake is about 15 mg, ten times less than the ADI (75).

There has been some concern that the feeding of EDTA compounds over long periods of time might affect the absorption of other micronutrients, such as zinc and copper. Calcium and magnesium would be unlikely to be affected since the amounts in the diet are, on a molar basis, many fold greater than the amounts of NaFeEDTA that would be used for fortification (75). The actual effects of EDTA compounds on zinc and copper metabolism have been studied in several animal studies and have shown an increase rather than a decrease in absorption and retention when added to diets of low bioavailability (75). Similar conclusions have been reached in a recent study in human subjects in which the effects of NaFeEDTA on the absorption of zinc and calcium were measured (78). It was concluded that the use of NaFeEDTA in populations consuming staple inhibitory diets would not only improve iron but also zinc nutrition. In contrast, calcium metabolism would not be affected. The concern that the administration of NaFeEDTA might increase the absorption of potentially toxic elements, such as lead, mercury, aluminum and manganese has recently been partially addressed. Manganese absorption and excretion were found to be unaffected by NaFeEDTA in human studies (79).

In contrast to the other salts of EDTA, NaFeEDTA was only recently recognised by JECFA as a food additive. Provisional approval was, given by JECFA in 1993 for its use in supervised food fortification programs in populations in which iron-deficiency anemia is endemic (80). At the time, JECFA requested further animal toxicological data and these were subsequently provided by the International Nutritional Anemia Consultative Group. As a result, in 1999 JECFA concluded that NaFeEDTA could be considered safe when used in supervised food fortification programs in response to a need for iron supplementation of the diet of a population as determined by public health officials. Such programs would provide daily iron intakes of approximately 0.2 mg/kg bw (81).

Cost considerations

While NaFeEDTA is currently six times more expensive than ferrous sulfate, it is twice as well absorbed. There is now

a need for food-grade NaFeEDTA to become more widely available and affordable (56). In this context, it seems entirely possible that the cost will drop if there is a large demand for fortification grade NaFeEDTA. Alternative strategies to reduce costs might include the use of ferrous fumarate together with Na_2EDTA in a 2:1 ratio; it is a ratio which has proved effective in radioisotopic absorption studies (73). One final point to be born in mind is the expectation that an effective fortification program would reduce after several years the costs of current supplementation and therapeutic programs for the control of iron deficiency (29).

FINAL COMMENTS

Iron fortification is an important component in any overall strategy to control iron deficiency anemia but before any comprehensive program is developed there should be an understanding of the extent and severity of the problem and its causes. It is a particularly attractive option where the intake of bioavailable iron is low but it should not be seen in isolation but rather as one part of a multipronged approach involving other complementary strategies, such as iron supplementation, dietary modification and the elimination of hookworm infestation. In operational terms, it is essential to identify the particular segments of the population which are to be the major targets of the program and then to choose suitable iron sources and vehicles to reach these targets. For this to be successful, active partnerships must be built up between many sectors, both public and private. In this context, government departments, private industry, the scientific community, the media, non-governmental organisations, consumer groups and donors all have important roles to play (59). In addition, such a coordinated program must be firmly embedded within the primary health care system and must address not only iron deficiency but also other micronutrient deficiencies. The comparative lack of success of fortification programs in many developing countries thus far can be ascribed to a number of factors but a central one has been a lack of commitment on the part of governments and the food industry to deal with the problem of iron deficiency in general and fortification in particular in a coordinated way (59). For success in the future, it would seem necessary for governments in developing countries to mandate fortification and to back this mandate with political will (59).

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Iron deficiency and neural development: An update

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SUMMARY. In Latin America, 10-30% of reproductive age females and upwards of 40-70% of pregnant women may be iron deficient. The true prevalence in young children and infants is often hard to determine because of problems in survey design, data collection, or sampling. There is little doubt, however, that iron deficiency anemia is a significant nutritional problem in many infants within the first 5 years of life. Numerous intervention studies have been performed across the world with varying success and it is clear that in nearly all situations it is a preventable disease with preventable consequences. One such consequence is the alteration in cognition that occurs in iron deficient individuals during the early parts of their life cycle and perhaps at later times as well. While iron deficiency was once presumed to exert most of its deleterious effects only if anemia was present, it is now clear that many organs show morphologic, physiologic, and biochemical changes before there is any drop in hemoglobin concentration. Iron deficiency is associated with alterations in many metabolic processes that may impact brain functioning; among them are mitochondria electron transport, neurotransmitter synthesis and degradation, protein synthesis, organogenesis, and others. It is necessary to separate the developmental aspects of iron deficiency and neural functioning from the aspects of iron deficiency that could occur at any time in life. A number of reviews have discussed the links between brain iron and neuropathology, brain iron, nutrition, and development, and iron status and cognition. New knowledge concerning the acquisition of iron by the brain in early life is being generated by numerous research groups. In the next decade a much clearer understanding of the role of brain iron on neural functioning will probably emerge.
Keywords: Iron deficiency, cognition, brain iron, neural functioning.

RESUMEN. Deficiencia de hierro y desarrollo neural: Una visión actual. En América Latina del 10-30% de las mujeres en edad reproductiva y por encima del 40-70% de las mujeres embarazadas pueden tener deficiencia de hierro. La verdadera prevalencia en lactantes y niños es difícil de determinar por problemas de diseño de encuestas, recolección de datos y muestreo. Sin embargo, existe poca duda de que la anemia por deficiencia de hierro es un problema significativo en muchos niños menores de 5 años. Numerosas intervenciones se han realizado con variados grados de éxito y es claro que casi todas las intervenciones la deficiencia de hierro es prevenible al igual que sus consecuencias. Una de estas consecuencias es la alteración en el desarrollo cognoscitivo que ocurre en individuos deficientes en hierro durante los primeros períodos de su vida y quizás posteriormente también. Aunque se creía que la deficiencia de hierro era más dañina sólo en presencia de anemia, es claro ahora que muchos órganos muestran cambios morfológicos, fisiológicos y bioquímicos antes de la disminución en los niveles de hemoglobina. La deficiencia de hierro está asociada a alteraciones en muchos procesos metabólicos que afectan el funcionamiento cerebral, como el transporte de electrones en la mitocondria, la síntesis y degradación de neurotransmisores, síntesis de proteínas, organogénesis y otros. Es necesario separar los aspectos de desarrollo de la deficiencia de hierro y el funcionamiento neural de los aspectos relacionados con la deficiencia de hierro que ocurren en cualquier momento de la vida. Varias revisiones han discutido la relación entre el hierro cerebral y las neuropatologías, hierro cerebral, nutrición y desarrollo y hierro y aspectos cognoscitivos. Continúan surgiendo nuevos conocimientos acerca de la adquisición de hierro por el cerebro en edades tempranas y se espera que en la próxima década se tenga un mejor entendimiento acerca del hierro cerebral y el funcionamiento neural.
Palabras clave: Deficiencia de hierro, cognitivo, funcionamiento neural, hierro cerebral.

Iron deficiency is the most common single nutrient deficiency in the world. It affects the lives of more than 2.1 billion people, with estimates of more than 50% of women of reproductive age and similar percentages of adolescents being iron deficient (1). In Latin America, 10-30% of reproductive age females and upwards of 40-70% of pregnant women may be iron deficient. The true prevalence in young children and infants is often hard to determine because of problems in survey design, data collection, or sampling. Numerous intervention studies have been performed across the world with varying success and it is clear that in nearly all situations it is a preventable disease with preventable consequences. One such consequence is the alteration in cognition that occurs in iron deficient individuals during the early parts of their life cycle and perhaps at later times as well (2). There is a history of nearly 30 years of efforts to document the effects of iron deficiency anemia on developmental delays in young children and infants (3-6).

Iron needs of the brain vary with the stage of the life cycle and the cell types that inhabit the Central Nervous System.

Iron is the key component of the many enzymes that involve essential oxidation-reduction reactions, synthesis of neurotransmitters, catabolism of neurotransmitters, and synthetic processes such as the production of myelin (60). While it is common to think about iron metabolism in the brain from a nutritional perspective, it is important to recall that iron is toxic to cells in the free state and several neuropathologies appear to have an iron accumulation component. One recent example of this is the disease, Friedrichís ataxia. In this disease there is an excess accumulation of iron in the mitochondria with a resulting neuronal death. It is believed that the fundamental defect is the failure of a frataxin gene product to be appropriately expressed resulting in poor transport of iron out of the mitochondria (64).

Acquisition of iron: The brain likely obtains iron via transferrin receptors expressed in endothelial cells on the brain microvasculature (7, 8). The movement of iron across this blood-brain barrier is likely affected by the iron status of the endothelial cells that comprise the barrier, as well as the astrocytes on the basolateral surface of the microvascular bed (55). The regulation of iron movement across this barrier is not well understood. The rate of iron uptake into the brain is affected by the iron status of the animal, increasing when the iron status is low and vice versa (9). In addition, the process is highly selective and not reflective of overall blood brain permeability (10,11). The uptake of iron is reported to be homogeneous followed by a redistribution of iron to the basal ganglia (12). Regions of the brain rich in iron in adulthood (i.e.: substantia nigra) are far less affected by iron deficiency than are other regions like the cortex or the striatum (13). Autoradiographic studies reveal a heterogeneous distribution of transferrin receptors in the adult brain (14,15), though studies of the relationship of this distribution to systemic iron status have not been published. The regional heterogeneity in the deposition of iron in the brain is remarkably similar across many species with the basal ganglia, substantia nigra, and deep cerebeliar nuclei particularly rich in iron (16). When magnetic resonance imaging was used to map iron distribution in the brains of children and adolescents, the highest concentrations of iron were found in globus pallidus, caudate nucleus, putamen, and substantia nigra (17). Recent studies from our laboratory and those of associates now demonstrate that the rate of iron accumulation in different brain regions is a function of the stage of brain development that is occurring at the time of the investigation (58). For instance, when brain iron distribution is studied in a rodent model of lactational iron deficiency; an entirely different pattern of iron loss emerges compared to the effects of iron deficiency instituted during the post-weaning period. Thus the needs for iron in the brain are very developmentally bound in early life with clear ramifications in functioning of some brain regions while having little impact in others. Recent studies in our laboratory demonstrated that pre and post-weanling iron deficiency result in very different patterns of iron loss in different brain regions (60,58). In the lactational iron deficiency period (equivalent to humans between 6-12 months of life); there is a very significant 25% drop in cortex, striatum, and hind-brain iron content. In contrast, there is only a 5% drop in thalamus iron content. During post-weanling iron deficiency there are comparable 20-30% declines in cortex, striatum, and hind-brain, but now, the thalamus also becomes sensitive to dietary iron deficiency and demonstrates a 20% drop in iron concentration. These studies demonstrate that the impact of iron deficiency on brain iron content is dependent on the timing of the nutritional insult compared to the brain growth occurring at that time.

Iron and transferrin levels have to been reported to be high in cerebrospinal fluid (CSF), especially in perinatal brains (18). The actual levels of iron in CSF however are poorly described in conditions of iron overload, iron deficiency, or during active growth and development. Our experience using atomic absorption spectrophotometry reveals CSF iron concentrations of approximately 15-25 ug/L in humans and monkeys, and 5-20 ug/L in mice (59,62). These concentrations are about 5-10 fold lower than the corresponding plasma concentrations. The choroid plexus is a rich source of transferrin mRNA, and transferrin is secreted by this organ presumably for use in the distribution of iron to glia and neurons for use or storage (19). The normal circulating level of Tf in CSF is also poorly described, though some reviews of the literature suggest the total iron binding capacity (TIBC) of the CSF is barely even with the circulating CSF iron concentrations resulting in the apparent availability of "free iron" (62). Our own experience with measurements of Tf and TIBC in CSF of humans, monkeys, and rodents however do not support this contention (unpublished data). The role of the CSF in the delivery of iron to various brain cells is not well understood. Iron appears first in the choroid plexus in some studies, and then re-distributes to other regions of the brain (63,61). Movement of iron from the choroid plexus to other parts of the brain imply a role for Tf, and perhaps other iron containing proteins, in this re-distribution (57). The exact identity, and mechanism of regulation in response to changing iron needs is being actively investigated by several laboratories at this time.

The predominant cell type containing iron in the mouse, rat, monkey, pig, and human brain is the oligodendrocyte (20). These cells are responsible for the production of myelin and hence alterations in the functioning of these cells are associated with hypomyelination. When oligodendrocyte maturation is disrupted, as occurs in myelin genetic mutants, iron accumulation is only about 50% of normal (21). In iron deficiency oligodendrocytes appear more "immature" (13).

Brain transferrin is made by oligodendrocytes and choroid plexus, although early in life it is likely derived from the plasma pool, since the blood-brain-barrier is not complete and the transferrin mRNA expression in these cells is low at that time (4). While there is no quantitative data to show that iron

deficiency leads to a lesser number of oligodendrocytes, hypomyelination occurs as a result of post-natal iron deficiency. Transferrin levels in the brain and cerebrospinal fluid fall from birth through 2 years of age, and can be affected by alterations in oligodendrocyte function (22). Rats exposed to a low iron diet after weaning have a doubling of brain transferrin content within 14 days of dietary iron restriction, reflecting a need for increased iron delivery and associated with lowered brain iron levels (13,23).

Ferritin consists of 24 protein subunits made up of different ratios of two isoforms; the L chain (19kD) and the H chain (21kD), to form a 450kD protein capable of binding in excess of 4000 atoms of iron. The brain ferritin is richer in the H isoform than in the L, and its localization is cell type specific (8,24). In rats, microglia and oligodendrocytes contain ferritin, whereas in mice astrocytes are ferritin positive. Ferritin levels correlate with brain iron content, and are highest at birth and decline thereafter in the newborn rat (25). Moreover, the concentration can be directly affected by the body iron burden (23). Ferritin isoforms are heterogeneously distributed in brain and seemingly, not all regions of the brain are equally sensitive to an alteration in body iron status (13,26). A recent study in young rats demonstrated that there is a shift in ferritin containing cell types in early post-natal life. During the first several days of post natal life the predominate cell containing ferritin are microglia and then soon after weaning, the predominate brain cells containing ferritin become the oligodendrocytes. H-ferritin, but not L-ferritin, was present in neuronal nuclei in the cortex (54). These data suggest that microglia play a role in iron homeostasis during early brain development and perhaps complete with oligodendrocytes and thus modulate the myelination processing of the oligodendrocytes. Studies in post natal iron deficiency involving H:L ferritin ratios in pig brain reveal a dramatic effect of iron deficiency an the expression of these two subunits of the ferritin molecule (56). The developmental roles of the two subunits, relative to iron storage or utilization and detoxification are unknown, although accumulation of iron in certain brain regions is believed to play a role in a number of neuropathologies (53).

Iron deficiency and neural functioning in humans: While iron deficiency was once presumed to exert most of its deleterious effects only if anemia was present, it is now clear that many organs show morphologic, physiologic, and biochemical changes before there is any drop in hemoglobin concentration (3,4,28). Iron deficiency is associated with alterations in many metabolic processes that may impact brain functioning; among them are mitochondria electron transport, neurotransmitter synthesis and degradation, protein synthesis, organogenesis, and others. Thus, it is not reasonable to assume that only very young infants or children are susceptible to the ill effects of iron deficiency. Indeed, in a report published in Lancet in 1996 non-anemic iron deficient adolescents had significant alterations in both memory (spatial) and attentional functions that was repaired with iron therapy (29). The animal studies of brain iron content during development demonstrate that iron deficiency in early life (prior to weaning) likely has long term effects that may be only partially reversible (30). In contrast, iron deficiency that occurs later in life (the post-weaning model) also had severe effects but they were readily reversible and of a different nature (13,31,32). Thus, the timing of iron deficiency seems critical to the severity and reversibility of its impact.

Observations of altered mental and motor development have been repeatedly made in young iron deficient children (5,33,34,50,51). Both the severity and the duration of the deficiency have been noted as significant factors (35). The earliest studies of Oski and colleagues (36) observed significantly lower Bayley developmental scores in iron deficient than control infants. The scores of iron deficient children were corrected within a short period of iron treatment. This theme of alterations in mental development and behavior as a function of early life iron deficiency was expanded upon by Lozoff and colleagues in several studies (5,37,38) as well as by Walter and colleagues (6,39). With moderate anemia, many of the children were considered to have abnormal affective behavior; in severe anemia (< 9 g/dL), all of the children were classified as abnormal. Lozoff further notes a decrease in close contact between anemic children and their mothers. This may be a manifestation of their affect, energy level, and voluntary activity (33). Since cognitive and behavioral development are strongly affected by the amount and level of environmental stimulation, this indirect route of impact should not be ignored.

Importantly, the studies of Lozoff as well as those of Walter (5, 6, 37-39) note a failure to improve performance in many of the anemic children after iron therapy despite hematologic normalization. In 1993, the research team headed by Pollitt demonstrated a normalization of developmental scores in infants who were anemic by providing a vigorous iron intervention for 4 months (40). While the reversibility of cognitive as opposed to developmental aspects of behavior have yet to be demonstrated, this study provided encouraging evidence for the benefit of improving iron nutritional status in infants.

Lozoff and colleagues have been interested in the possibility that iron deficient anemic infants are "functionally isolated" (52). The behavior of 52 Costa Rican 12-23 -month-old infants with iron deficiency anemia was contrasted with that of 139 control infants with better iron status. The investigators observed the infants during free play and also tested the infants with standardized motor and mental protocols. Infants with iron deficiency were more wary, hesitant, and easily tired; made fewer attempts at test items, were less attentive to instructions, and were less playful. In addition, the adults around the iron deficient infants interacted with them less. These data support the hypothesis that iron deficient anemic

infants engage less with their environment and in return, receive less stimulation from their environment. While this model also argues for the possibility of a generalized "nutritional stress" associated with decreased affect and activity, it does not exclude the possibility that direct biologic effects of iron deficiency may result in these outcomes. The task of assigning clear roles for iron deficiency anemia in developmental delays or specific cognitive tasks is difficult (49).

Neurobiological sequelae of iron deficiency: When animals are given low iron diets in post-weaning life, there is a significant decline in brain iron content and a rapid repletion with refeeding (13,23). This is in contrast to neonatal or pre-weaning iron deficiency in which the effects appear irreversible (30,41,42). Based on animal studies across a number of species we assume that human brain iron content goes down with a decrease in body iron status, although there is no direct proof of this.

Iron is required for proper myelination of the spinal cord and white matter of cerebellar folds (43) and it is a co-factor for a number of enzymes involved in neurotransmitter synthesis including tryptophan hydroxylase (serotonin) and tyrosine hydroxylase (norepinephrine and dopamine). Iron is also a co-factor for ribonucleotide reductase, the rate limiting step in DNA synthesis. Thus, it is easy to postulate that deprivation of iron to the brain during periods of very active myelination could result in poorly functioning neurons. Dr. Walter reported at the International Nutrition Society meetings in August of 1997 that iron deficient infants have a decreased nerve conduction velocity when auditory evoked potential studies are conducted, an observation consistent with improper myelination.

To date, the dopaminergic system in the only neurotransmitter system in the CNS that has been consistently responsive to experimental changes in iron status. As whole brain iron content drops 15% below normal, biologic and behavioral alterations occur as a result of changes in the dopaminergic system (44,45). These scientists measured affinities and densities for dopamine D1 and D2 receptors, serotonin, γ-aminobutyric acid, benzodiazepine, α and ß adrenergic, and muscarinic-cholinergic receptors in brain regions after post-weaning dietary iron deficiency. Iron is colocalized with dopaminergic neurons (20,46) and cell bodies throughout the brain with a lesser colocalization with γ-amino butyric acid. There are four major dopaminergic tracts in the brain. Two of these tracts pass from the substantia nigra to and through the caudate nucleus and putamen (also called the striatum). It is precisely in these areas that iron is in highest concentration, and that neurobiologic changes occur when dietary iron deficiency is created. Recent in vivo animal data demonstrate that extracellular dopamine is elevated in iron deficiency and it returns to normal levels when brain iron content and iron status return to normal (47). Attentional processing of environmental information is highly dependent on appropriate rates of dopamine clearance from the interstitial space, which suggests that iron status may be affecting behavior through effects on dopamine metabolism. Alterations in dopamine in the mesolimbic and the nigrostriatal tracts are associated with changes in motor control as well as altered perception, memory, and motivation. The loss of affect, arousal, and perception are often the characteristics that investigators associate with iron deficient infants, children, and adolescents (40).

Irreversible alterations in brain iron content have been shown in animal studies by feeding rats low iron diets early in life, prior to the completion of the brain organization, myelination, and the establishment of the dopaminergic tracts (42, 48). A significant caveat to the observations from these rodent studies is that much of the rodent brain maturation occurs post-natal. However, in species like humans whose brain growth is slower and spans significant pre-natal and post-natal periods, the sensitivity of various brain processes to the nutritional insult may be different.

In summary: A number of reviews have discussed the links between brain iron and neuropathology (27), brain iron, nutrition, and development (2-4), and iron status and cognition (39). We are still ignorant about many of the biologic details concerning the relationship of body iron status, development, and brain functioning, and most importantly we are severely lacking neurobiologic explanations for the consistent changes in attentional processes in young people. Dopamine biology may be significant, but other neurotransmitter and neuropeptide systems not yet thoroughly examined may also play very significant roles. New knowledge concerning the acquisition of iron by the brain in early life is being generated by numerous research groups. We are hopeful that in the next decade a much clearer understanding of the role of brain iron on neural functioning will emerge.

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Participación del hierro en la inmunidad y su relación con las infecciones

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RESUMEN. Las evidencias experimentales acumuladas en los últimos años demuestran que el hierro es un elemento fundamental para el normal desarrollo del sistema inmunitario y para su adecuado funcionamiento, de manera que su deficiencia afecta profundamente la capacidad del sistema de montar una efectiva respuesta. El papel que el hierro desempeña en la inmunidad se manifiesta en primer lugar, dentro de los procesos de inmunidad innata. El hierro es un elemento necesario para la proliferación y maduración de las células inmunitarias, particularmente de los linfocitos, asociados con la generación de una respuesta específica frente a agentes infecciosos. El organismo tiene la capacidad a través de proteínas tales como la transferrina y la lactoferrina de reducir la disponibilidad de hierro para consumo por elementos infecciosos.
El hierro es también un elemento esencial para la proliferación de muchas bacterias, parásitos y células neoplásicas, por lo cual, un exceso de hierro en el organismo potencialmente facilitaría el desarrollo de infecciones y la invasión por células tumorales. El sistema inmunitario posee mecanismos bacteriostáticos que reducen la disponibilidad del metal, interfiriendo así con el crecimiento bacteriano, y además utiliza el hierro como intermediario en la producción de moléculas bactericidas.
Palabras clave: Hierro, inmunidad, deficiencia de hierro, infección.

SUMMARY. Role of iron in immunity and infection. Experimental evidence in the last decades show that iron is a fundamental element for normal development of the immune system. Its deficiency affects the capacity to have an adequate immune response. The role of iron in immunity is necessary for immune cells proliferation and maturation, particularly linphocytes, associated with the generation of a specific response to infection. The body has the capacity to reduce the iron availability to be consumed by infectious elements by proteins such as transferrin and lactoferrin. Also, iron is essential for the proliferation of bacteria, parasites, and neoplastic cells. Thus excess iron could potentially facilitate the development of infections and the invasion of tumoral cells. The immune system has bacteriostatic mechanisms that reduce the availability of the metal, interfering with bacterial growth. Additionally the system uses iron as the intermediary in the production of bacteriostatic cells.
Keywords: Iron deficiency, immunity, infection.

INTRODUCCION

El hierro es un elemento fundamental para el hombre, no sólo desde el punto de vista económico, cultural, social, e histórico, sino también desde una perspectiva biológica. En este último caso, ha sido claramente establecida la vital importancia de este metal para el crecimiento y desarrollo humano, además de una gran variedad de especies biológicas, inclusive las más inferiores como las bacterias. La importancia del metal se refleja en la gran atención que se ha dedicado a su estudio, en virtud de lo cual se ha acumulado una enorme masa de conocimientos sobre su función biológica. Las alteraciones en su metabolismo, particularmente su deficiencia, están consideradas entre los trastornos nutricionales más frecuentes en el mundo, tanto en países desarrollados como en aquellos llamados, eufemísticamente, en vías de desarrollo. La principal consecuencia de una reducida utilización de este metal por el organismo es la anemia por deficiencia de hierro, con sus diversos grados de severidad. Además se producen otras anormalidades tales como alteraciones en el crecimiento y desarrollo, retardo en la maduración de las capacidades intelectuales y neurológicas, trastornos en el epitelio gastrointestinal, y alteraciones en diversos componente inmunitarios. Asimismo, la sobrecarga o exceso de hierro también produce importantes trastornos orgánicos y afecta igualmente el sistema inmunitario.

ABC de la química biológica del hierro

El hierro se clasifica químicamente como un metal de transición; su estructura orbital (electrónica) le permite cambiar fácilmente su estado de oxidación mediante la pérdida o ganancia de un electrón, por lo cual se encuentra bajo dos formas iónicas: la ferrosa (Fe^{2+}) y la férrica (Fe^{3+}). Esta característica le confiere excelentes propiedades para partici-

par en los procesos biológicos de oxido-reducción (transferencia de electrones), de gran importancia para el metabolismo celular. A su vez, lo convierten en un peligroso metal para catalizar la formación de intermediarios inestables, llamados también radicales libres, los cuales son altamente tóxicos (1). Los daños celulares inducidos por estos radicales resultan de la peroxidación de los lípidos de las membranas, de la ruptura del ADN, de la inactivación de diversas enzimas (i.e., de la vía glicolítica, de la cadena respiratoria, etcétera), y por alteración de los reservorios de calcio. Toda una espada de doble filo.

En los sistemas biológicos el hierro suele encontrarse unido a proteínas llamadas metaloproteínas férricas, las cuales pueden clasificarse en tres grandes grupos:

- Las que forman complejos reversibles con Fe (ferritina, la transferrina y lactoferrina).
- Las que tienen capacidad de combinarse reversiblemente con el oxígeno (hemoglobina y mioglobina).
- Las que tienen función enzimática, especialmente las que participan en funciones de óxido-reducción (redox), (citocromos y ribonucleótido-reductasas).

Hierro e inmunidad

El papel que el hierro desempeña en la inmunidad se manifiesta en tres aspectos fundamentales. En primer lugar, dentro de los procesos de inmunidad innata (llamada también primera línea de defensa inmunitaria) parte de los mecanismos bactericidas y bacteriostáticos dependen del funcionamiento de moléculas férricas; en segundo lugar, es un elemento necesario para la proliferación y maduración de las células inmunitarias, particularmente de los linfocitos, asociados con la generación de una respuesta específica frente a agentes infecciosos. El tercer aspecto es la capacidad que tiene el organismo, a través de proteínas tales como la transferrina y la lactoferrina de reducir la disponibilidad de hierro para consumo por elementos infecciosos.

Las primeras indicaciones de la participación del hierro y de las proteínas asociadas o relacionadas con él en el proceso inmunitario, se derivan de las observaciones hechas en 1946 por Schade y Caroline (2). Estos investigadores fueron los primeros en determinar la presencia en el plasma humano de transferrina (TF) y de demostrar su capacidad para interferir con los procesos de proliferación bacteriana (efecto bacteriostático) *in vitro*. Si bien se considera que la TF es fundamentalmente una proteína transportadora de hierro su verdadero papel como componente antibacteriano *in vivo* es materia de discusión. En este sentido, el papel de la lactoferrina (LF) está mejor establecido. La LF es una proteína captadora de hierro que posee una afinidad por el metal 300 veces mayor que la de la transferrina. La mayor parte se encuentra en las secreciones orgánicas externas (saliva, leche, secreciones gastrointestinales, seminales y vaginales, lágrimas, etcétera), donde usualmente existe una flora bacteriana, cuyo desarrollo suele estar controlado o limitado (3). Por ejemplo, la presencia de LF en la saliva limita el crecimiento del *Actinobacillus actinomycetens comitans*, un agente potencialmente patógeno involucrado en la producción de periodontitis (4); por otra parte, se cree que el incremento de los niveles de LF que se observa en pacientes con fibrosis quística puede contribuir a la menor incidencia de caries que se observa en estos pacientes (5). La concentración de LF en el plasma y en el líquido cefaloraquídeo es baja, pero se incrementa en procesos inflamatorios, por lo cual se la ha considerado como una de las proteínas de fase aguda. En este caso, el incremento de concentración es debido a su secreción a partir de los gránulos de los neutrófilos. Recientemente se ha identificado en ratones una proteína denominada Nramp 1 (Natural resistance-associated macrophage protein), la cual es codificada por el gen *Bcg*, que confiere resistencia a la infección con gérmenes tales como micobacterias, leishmanias y salmonelas. Precisamente dicha proteína está presente en la membrana de los lisosomas macrofágicos, y aunque su función no se conoce con exactitud, se ha propuesto que actúa reduciendo los niveles de hierro intracelular, el cual sería transportado hacia los fagolisosomas donde se utilizaría para catalizar la producción de radicales libres tóxicos para los gérmenes fagocitados (6).

Deficiencia de hierro e inmunidad celular y humoral

La importancia del hierro en la inmunidad se pone de manifiesto sobre todo cuando existe una deficiencia del metal. Es ampliamente conocido que la deficiencia de hierro afecta de una manera notable a la eritropoyesis, reflejando de esa manera, el mayor requerimiento de ese proceso, puesto que alrededor del 70% del hierro es utilizado en la formación de hemoglobina. Pero además, también afecta otros sistemas, notablemente el sistema nervioso, el sistema inmunitario y las mucosas .

Las alteraciones inmunitarias celulares asociadas con la deficiencia de hierro en estudios clínicos son variadas: reducción de la reacción de hipersensibilidad tardía frente a diversos antígenos (7,8), disminución de la capacidad proliferativa de linfocitos en cultivo en respuesta a mitógenos y antígenos (9,10), y reducción del número de linfocitos T circulantes (11). Estas observaciones han sido confirmadas en modelos experimentales en ratas, ratones y cobayos, en donde además se ha observado una disminución en la celularidad de órganos linfoides, tales como bazo, timo y ganglios linfáticos, además de alteraciones de la linfopoyesis esplénica y tímica, y en la estructura organizativa de estos órganos en ratas lactantes, y en general una disminuída capacidad de respuesta. Es posible que todos estas observaciones puedan explicarse por una disminución en la capacidad proliferativa de las células linfoides debido a una reducción en la actividad de la enzima ribonucleótido reductasa, cuya consecuencia es una reducción en la síntesis de ADN.

Con respecto a la inmunidad natural mediada por células NK (asesinas), en ratas y ratones que han desarrollado tumores malignos y a la vez son deficientes de Fe, se ha demostrado una

reducción en la actividad citolítica de estas células. Igualmente la función secretoria de los macrófagos con relación a ciertas citoquinas, tales como MIF (Factor Inhibitorio de Migración de Macrófagos), interferón e interleukina 1 también está afectada. El interferón es un potente activador de la actividad de linfocitos NK y de otros macrófagos, no sólo contra células tumorales, sino también contra células infectadas por virus, y la IL-1 es una molécula importante en la regulación de la respuesta de fase aguda ante las infecciones. El MIF es importante en el proceso de generación de la hipersensibilidad tardía. Por su parte, el componente humoral de la respuesta inmunitaria parece afectarse menos por la deficiencia de hierro. Varios estudios sobre el nivel de inmunoglobulinas totales o anticuerpos específicos en suero no han podido demostrar un efecto consistente en pacientes deficientes (7,12). La producción de anticuerpos antitetánicos y antidiftéricos se ha encontrado normal o discretamente elevada, cuando se estudia su nivel basal o luego de un reto antigénico. En ratas deficientes de hierro los niveles de IgA (la inmunoglobulina secretoria) en saliva, leche y suero no están alterados. Sin embargo, en otros experimentos, en los cuales se han usado ratas lactantes y técnicas más sensibles para medir la producción de anticuerpos, se ha demostrado una disminución en la repuesta humoral frente a un reto con eritrocitos de carnero. Es interesante notar que en estos modelos experimentales la corrección de la deficiencia férrica no mejoró a corto plazo la respuesta inmunitaria.

Con relación a la inmunidad innata, algunos autores han reportado una disminución del número de fagocitos circulantes y una reducción de la capacidad bactericida de los neutrófilos (13). En ratas lactantes, otros autores han reportado un incremento en el número de neutrófilos circulantes, pero la actividad fagocítica per se, corregida para el número de células, está disminuída significativamente. Esto se ha interpretado como una migración de granulocitos no totalmente competentes de la médula ósea hacia la sangre.

También se han reportado alteraciones en la producción de ciertas citoquinas, tales como IL-2 (14) y TNF- (15). Sin embargo, Bhaskaram et al. (16) reportaron una secreción normal de IL-1 por macrófagos de niños deficientes en hierro.

Estudios experimentales in vitro

Diversos estudios *in vitro* han demostrado claramente que el hierro es captado por linfocitos a partir del medio de cultivo durante el proceso de proliferación estimulado por mitógenos o antígenos (17-19). A su vez, tal efecto queda confirmado en experimentos donde se demuestra que la adición de desferrioxamina, un potente quelante de hierro, inhibe la proliferación linfocitaria inducida por mitógenos del tipo de la fitohemaglutinina (20,21). De hecho, durante la activación linfocitaria uno de los eventos que ocurre más tempranamente es la expresión de receptores de transferrina, mediante los cuales los linfocitos internalizan transferrina, de la cual toman el hierro que requieren para sus procesos metabólicos. La ausencia de transferrina de medios de cultivo interfiere con la activación linfocitaria.

Numerosos estudios *in vitro* han señalado el efecto inhibitorio que sales de hierro, particularmente el citrato férrico, ejercen sobre diferentes funciones y parámetros del sistema inmunitario: fagocitosis por polimorfonucleares humanos (22,23) o de conejos (24), capacidad tumoricida de macrófagos múridos (25,26), disminución de la formación de rosetas por parte de linfocitos T (19,27), respuesta proliferativa ante estímulos mitogénicos (18,19,28), reacción mixta de linfocitos (29), actividad de células NK (26), y proporción de linfocitos CD4+ (30). En estos casos hay que recalcar que si bien tales observaciones son consistentes y reproducibles, tales efectos no se deben a un efecto directo del hierro en sus formas fisiológicas, sino a la formación de complejos polinucleares del metal que se forman en cultivo y que interfieren en forma no fisiológica con las funciones de las células inmunitarias (19). Estos resultados han sido comprobados por otros autores en diferentes modelos experimentales. Un efecto similar se logra mediante la adición de ferritina a los medios de cultivo (28,31), efecto inhibitorio que también se extiende a los procesos de mielopoyesis y eritropoyesis (32,33).

Estudios *in vitro* han demostrado un efecto inhibitorio de la deficiencia de hierro sobre el metabolismo celular (34), lo cual en cierta forma podría explicar la reducción en la capacidad proliferativa de las células inmunitarias observadas por otros autores. Kuvibidila et al. (35) han postulado que alteraciones en la activación de la proteinkinasa C podría ser uno de los posibles mecanismos de la disminuida proliferación linfocitaria observada en ratones deficientes de hierro.

Hierro e infección

La evidencia presentada anteriormente demuestra claramente que el hierro es fundamental para el desarrollo de una respuesta inmunitaria adecuada y totalmente efectiva, de manera que la deficiencia del metal interfiere con los mecanismos de defensa del organismo. Podría decirse que, bajo tales condiciones, la susceptibilidad a las infecciones estaría aumentada, pero para ello hay que tomar en cuenta también los requerimientos férricos de los agentes infecciosos involucrados. Se ha establecido que la gran mayoría de los agentes infecciosos requieren hierro para su proliferación, y para satisfacer esos requerimientos han desarrollado variados mecanismos que le permiten obtenerlo a partir del contenido tisular del huésped o de su medio ambiente. Las moléculas involucradas en estos procesos han recibido el nombre genérico de sideróforos, entre las cuales se conocen algunas como la desferrioxamina, que tiene uso terapéutico en el tratamiento de la sobrecarga de hierro. Por su parte, el huésped utiliza proteínas que le permiten mantener el hierro en forma no tóxica, tal es el caso de la ferritina y de la transferrina, y aún más, para hacerlo inasequible a los microorganismos, tal es el

caso de la lactoferrina. Por lo tanto, la aparición de un cuadro infeccioso clínico va a depender no solo del estado del sistema inmunitario, cuya reserva o capacidad es bastante grande, sino también del status férrico del individuo y de la patogenicidad de los microorganismos.

A) Estudios clínicos

Desde finales del siglo XIX se comenzó a establecer la existencia de una estrecha relación entre el status férrico de las personas y la susceptibilidad a ciertas infecciones. Trousseau (36) observó que el tratamiento con hierro de pacientes con tuberculosis inactiva frecuentemente producía una recurrencia clínica de la enfermedad. Algo similar fue observado en la década de 1970 en pacientes con malaria (37). Por otra parte, los primeros estudios que demuestran un efecto importante de la deficiencia de hierro sobre la susceptibilidad a las infecciones en niños datan de la década de 1920 (38), de donde surgió la recomendación de fortificar la leche bovina para uso infantil, lográndose una mejoría en las cifras de hemoglobina y hematocrito, y una reducción de 50% en la incidencia de infecciones respiratorias y gastrointestinales. Una plétora de estudios posteriores en condiciones clínicas tanto de deficiencia como de sobrecarga de hierro han arrojado resultados variables; por una parte, una gran mayoría apunta hacia la presencia de un incremento en la susceptibilidad a las infecciones, de mayor o menor grado, pero hay que apuntar que existen unos cuantos estudios en los cuales no se ha podido demostrar tal condición.

Paralelamente al incremento en la susceptibilidad a las infecciones, en otros estudios se ha demostrado una disminución en la actividad bactericida y bacteriostática de los neutrófilos, asociada con alteraciones en la actividad quimiotáctica y en la generación del estallido respiratorio, que es esencial para la generación de moléculas tóxicas para las bacterias. En general, tales alteraciones fueron revertidas luego de dos semanas de tratamiento con hierro oral o parenteral.

A lo largo de los años la relación deficiencia férrica - infección ha sido confirmada en numerosos estudios, pero a su vez han surgido algunos aspectos aparentemente paradójicos. Por un lado, se ha observado que determinadas poblaciones en las cuales se presenta una alta incidencia de anemia por deficiencia de hierro, también muestran una alta frecuencia de enfermedades infecciosas, asociada en muchos casos con alteraciones del sistema inmunitario; sin embargo, por otro lado, también se ha observado una elevada incidencia de infecciones en personas, particularmente en niños, que han recibido dosis elevadas de hierro oral o parenteral, y también en individuos que sufren de enfermedades asociadas con sobrecarga de hierro. La explicación de la aparente paradoja mencionada antes estriba en el delicado balance que se establece entre los requerimientos metálicos de los agentes infecciosos involucrados y los del huésped por este metal, que lo requiere para multitud de procesos fisiológicos, pero en este caso, particularmente para la generación de una adecuada respuesta inmunitaria. Debemos tomar en cuenta también que la deficiencia de hierro podría afectar mecanismos de defensa no inmunitarios tales como el normal mantenimiento de las barreras epiteliales y mucosas, y así favorecer la invasión de microorganismos. Por otra parte, en los casos de sobrecarga de hierro, otro aspecto que debe ser tomado en cuenta son las propiedades tóxicas del hierro, que afectarían negativamente las defensas del organismo.

Los numerosos estudios clínicos y epidemiológicos sobre la relación hierro e infección han provisto evidencias indirectas de la existencia de tal relación, aunque en algunos casos esas evidencias son variables, e incluso contradictorias, por lo cual, aunque son consistentes y abundantes, no han sido universalmente aceptadas, y de cierta manera son susceptibles a ciertas críticas. La principal se deriva del hecho de que, con enorme frecuencia, los individuos estudiados con deficiencia de hierro, tienen concomitantemente anemia, otras deficiencias nutricionales, tanto de micronutrientes (por ejemplo Zn o Cu) como proteico-calórica, además de diversas parasitosis. Debe también tomarse en cuenta que, cuando existe deficiencia de Fe, la absorción de ciertos metales tóxicos como el plomo (Pb) suele estar aumentada, lo cual contribuye a oscurecer más el panorama. Por todo lo anterior es difícil concluir de estos estudios, que es el hierro el único o el principal responsable de los trastornos observados. Además, los estudios clínicos y epidemiológicos a veces no pueden ser comparados entre sí debido a las diferencias en los tipos de poblaciones estudiadas, status socioeconómico, diseño experimental y metodología utilizada; por otra parte, tales estudios no dejan de ser simplemente descriptivos, de manera que las alteraciones moleculares y celulares inducidos por o asociados con los desbalances férricos no han sido totalmente clarificados en esos sistemas. Por esa razón, muchos investigadores han recurrido a la experimentación animal *in vivo*, particularmente en ratas y ratones, y a la experimentación *in vitro*, que permite también el uso de células humanas.

B) Estudios experimentales

En modelos animales se ha demostrado que la deficiencia de hierro aumenta la susceptibilidad a las infecciones bacterianas. Por ejemplo, ratas deficientes de hierro son incapaces de eliminar adecuadamente una dosis infecciosa de *Salmonella typhimurium* administrada oralmente (39) y son menos resistentes a infecciones por estreptococos (40). Por otra parte reduce la respuesta de anticuerpos frente a una inmunización con toxoide tetánico (41).

En el caso de las parasitosis, la mayoría de los estudios se han enfocado hacia los protozoarios. En estudios clínicos se ha observado un incremento de la parasitemia después de administrar un suplemento de hierro a pacientes palúdicos (37). Paradójicamente, en infecciones experimentales con esporozoitos de *Plasmodium yoelii* en ratones deficientes de hierro se ha observado un mayor desarrollo del parásito en el hígado y una más temprana parasitemia (42), mientras que en

ratones infectados con *Trypanosoma cruzi* se produce un incremento de la parasitemia después de la inyección de hierro dextrán (43). En el modelo múrido de infección con *Ascaris suun*, una dicta deficiente de hierro no influencia la carga parasitaria pero si la resistencia a infección luego de una inmunización (44). Estudios realizados en nuestro laboratorio en ratones deficientes de hierro infectados con larvas de *Schistosoma mansoni* demuestran que estos animales presentan una carga parasitaria reducida, así como una reducción del número y tamaño de los granulomas hepáticos, y una disminución de las inmunoglobulinas totales y de los anticuerpos dirigidos contra antígenos solubles del huevo. Además se evidenció una reducción en la proliferación linfocitaria y en la producción de interleuquina 2, aunque no de interleuquina 4 (45).

Sobrecarga de hierro, inmunidad e infección

La sobrecarga o exceso de hierro en el organismo también afecta la función inmunitaria. Por ejemplo, en pacientes con -talasemia major y hemosiderosis se ha demostrado una reducción en la actividad de las células NK, así como una disminución en la función fagocítica y bactericida de los neutrófilos sanguíneos en pacientes sobrecargados sometidos a diálisis (46,47). En pacientes con cáncer que reciben quimioterapia agresiva se produce una hiperferremia acompañada con aumento de la saturación de transferrina, lo cual se asocia con un incremento en la susceptibilidad a las infecciones. El incremento en la saturación de transferrina se ha asociado con un incremento en la tasa de crecimiento in vitro de ciertas bacterias tales como Escherichia coli y Staphylococcus aureus.

Tanto la transferrina como la lactoferrina tienen efecto bacteriostático *in vitro* para diferentes tipos de bacterias (48-50). En condiciones normales sólo un tercio de la transferrina sanguínea se encuentra saturada con hierro, lo que le confiere una gran capacidad para captar hierro libre de los líquidos biológicos. En condiciones de sobrecarga de hierro, la saturación de la transferrina llega a su mayor nivel, y esto compromete sus propiedades bacteriostáticas. Aunque la susceptibilidad a las infecciones no es la característica fundamental de la sobrecarga de hierro, se han descrito numerosos casos de infecciones en estos pacientes, a menudo debido a gérmenes poco comunes u oportunistas (48,51,52) (Tabla 1).

TABLA 1
Infecciones asociadas con sobrecarga de hierro

BACTERIANAS: *Listeria monocytogenes, Yersinia enterocolitica, Salmonella typhimurium, Klebsiella pneumoniae, Vibrio vulnificus, Escherichia coli.*
FUNGICAS: *Cunninghamella bertholeatiae, Rhizopus oryzae, Mucor sp.*

Hierro, inmunidad y neoplasia

Muchos de los conceptos establecidos hasta ahora con respecto a la relación hierro, inmunidad e infección, pueden aplicarse en el caso de neoplasia, considerada ésta como un agente o elemento invasivo o infeccioso (53-55). El hierro es un nutriente esencial para células tumorales, y muchas de ellas expresan receptores de transferrina en cantidad mayor que las células normales, además de usar hierro no unido a TF para diversas funciones metabólicas. El exceso de hierro podría también predisponer al desarrollo de ciertas neoplasias (56). Por otra parte, el hierro puede actuar como promotor de la invasión neoplásica a través de la inhibición de las defensas inmunitarias. Así por ejemplo, ha sido demostrado que el hierro es capaz de inhibir la actividad tumoricida de macrófagos (57) y también la actividad de linfocitos citolíticos naturales (las llamadas células NK o natural killers) (58).

Respuesta inmunitaria y metabolismo del hierro

Así como las alteraciones en el metabolismo del hierro tienen un efecto sobre el sistema inmunitario y su capacidad de responder frente a diversos estímulos, la respuesta inmunitaria, y particularmente la de tipo innata, es capaz de modular el metabolismo del hierro. Es así como, en pacientes que sufren de infecciones crónicas, tumores malignos avanzados y trastornos autoinmunitarios, suele observarse la llamada anemia de enfermedad crónica, que tiene las características de una anemia por deficiencia de hierro, pero cuya patogénesis no está clara (59,60). En el caso de infecciones crónicas y de tumores malignos, se cree que tal anemia resulta de una sobreestimulación del sistema inmunitario y una sostenida liberación de citoquinas, como el interferón gamma (IFN-), el cual, a través de la estimulación de la síntesis de NO, interfiere con el metabolismo del hierro (37,61). Esto se manifiesta en una reconducción del hierro hacia sus depósitos en el sistema retículo endotelial o fagocítico mononuclear, en un intento del sistema inmunitario de incrementar la actividad efectora de los fagocitos contra microorganismo o células tumorales. Es también posible que los altos niveles de ferritina que suelen verse en estas condiciones tenga un efecto inhibitorio sobre la eritropoyesis, como ha sido demostrado in vitro, contribuyendo así a la patogénesis de la anemia (28,33,60).

CONCLUSIONES

Las evidencias experimentales acumuladas en los últimos años demuestran que el hierro es un elemento fundamental para el normal desarrollo del sistema inmunitario y para su adecuado funcionamiento, de manera que su deficiencia afecta profundamente la capacidad del sistema de montar una efectiva respuesta. Por otra parte, el hierro es también un elemento esencial para la proliferación de muchas bacterias, parásitos y células neoplásicas, por lo cual, un exceso de hierro en el organismo potencialmente facilitaría el desarrollo de infecciones y la invasión por células tumorales. A su vez, el sistema inmunitario posee mecanismos bacteriostáticos que reducen la disponibilidad del metal, interfiriendo así con el

crecimiento bacteriano, y además utiliza el hierro como intermediario en la producción de moléculas bactericidas. Tal efecto se extiende además a la respuesta antitumoral.

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Nuevas alternativas en la prevención de la deficiencia de hierro. Uso de la ingeniería genética en la modificación de alimentos

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RESUMEN. Este artículo discute algunas de las posibles aplicaciones de técnicas de modificación genética para introducir algunos compuestos en plantas o animales. El potencial de estas técnicas de modificación de plantas es muy amplio y va desde mejoras en la producción de alimentos para consumo humano y para el desarrollo pecuario, pasando por la producción de anticuerpos o proteínas para uso terapéutico hasta las modificaciones que implican la inclusión de nutrientes para mejorar el valor nutritivo de un alimento y la producción de vacunas. Debe tenerse presente, sin embargo, que el alcance o las consecuencias de estas modificaciones no están totalmente dilucidadas. Hay preocupación por efectos secundarios o la aparición de nuevos virus por recombinación genética que no han sido totalmente descartados, aunque la tendencia es considerar el proceso como seguro. Finalmente se presentan evidencias sobre la posibilidad de introducir en vegetales la capacidad de sintetizar vitamina A o de producir arroz con un alto contenido de hierro como alternativas reales para combatir algunas de las deficiencias nutricionales mas importantes a nivel mundial.
Palabras clave: Biotecnología, modificación genética.

SUMMARY. New alternatives in the prevention of iron deficiency. Use of biotechnology in food modification. This article reviews the possible applications of new food biotechnology techniques to introduce some compounds into plants or animals. The potential for these plant modification methods has ample applications ranging from improvements in food production and development for human consumption, production of antibodies or therapeutic proteins, inclusion of nutrients to improve nutritional value of the food to production of vaccines. It must be clear though that currently the scope and consequences of such modifications are not completely clear. There is some concern about potential secondary effects and the hipothesis of the appeareance of new viruses due to recombinant genetical transformations that have not been totally rejected. However the tendency is towards considering the process as safe. Finally some evidence is presented about the possibility of introducing the capacity to sinthesize vitamin A in vegetables or produce rice with high content of iron as real alternatives to fight some of the nutritional deficiencies most common worldwide.
Keywords: Biotechnology, genetic modification, GMO.

Desde hace unos pocos años se ha abierto un nuevo campo de investigaciones y posibilidades con la manipulación de genes. Se han hecho realidad deleciones, transformaciones, inserciones y un sinnúmero de manipulaciones genéticas que hace 20 años parecían imposibles y que se traducen en mejoras a nuestra calidad de vida a pesar de no haber sido evaluados en su totalidad las limitaciones o posibles daños. En esta conferencia enumeraré algunos logros importantes en cuanto a manipulación genética con especial énfasis en alimentos transgénicos en general y las modificaciones que afectan de alguna manera el metabolismo de hierro.

Leche

La producción de algunas sustancias usadas en transfusiones (factores de coagulación, interleucinas, eritropoyetina) presenta ciertos inconvenientes como altos costos de producción industrial, requerimiento de purificaciones exhaustivas y rigurosas antes de poder ser usados por lo que el rendimiento es por lo general bastante bajo (1). Algunas estrategias recientes incluyen la obtención de estas proteínas a partir de la leche de animales transgénicos. Esto se logra introduciendo un vector de fusión en huevos fertilizados de cabras, ovejas o cerdos, que transmitirán el gen a su progenie. El producto se expresa solo en la leche debido a que el vector de fusión contiene secuencias regulatorias para proteínas específicas de la leche como caseína o lactoglobulina. Sin embargo, este procedimiento tiene algunos problemas como expresión no predecible, glicosilación alterada y la posible presencia de agentes patógenos.

Se ha logrado también producir lactoferrina humana (hLF) en leche de vaca (2). Para ello se usaron vectores de expresión específicos de glándula mamaria basados en elementos regulatorios de caseína bovina que contenían bien el cDNA de lactoferrina humana o secuencias genómicas de hLF. Dichos vectores fueron introducidos en líneas germinales bovinas por inyección pronuclear a embriones. Los niveles de lactoferrina humana fueron bajos en la leche de las vacas a las que se insertó el cDNA, pero del orden de miligramos por litro en la leche de las que contenían secuencias genómicas.

La expresión de lactoferrina humana no afectó la cantidad

y composición de la leche, ni estuvo asociada a cambios en la salud de las vacas. La lactoferrina recombinante resultó virtualmente idéntica a la lactoferrina humana por criterios inmunológicos, funcionales y estructurales. Los autores especulan que la lactoferrina recombinante tendría efectos antimicrobianos y antiinflamatorios similares a los reportados *in vivo*.

Plantas

Otro gran acierto en cuanto a manipulación genética ha sido la obtención de hemoglobina humana a partir de plantas de tabaco (3). Actualmente se obtienen los sustitutos sanguíneos basados en hemoglobina a partir de sangre bovina o humana descartada, expresando la hemoglobina en bacterias, levaduras o animales transgénicos con algunos problemas como oxidación del hemo y la presencia de agentes infecciosos. Las plantas transgénicas podrían ser una buena alternativa como recurso mas económico y menos susceptible a contaminación. En este trabajo se transformaron plantas de tabaco con *Agrobacterium tumefaciens* que contenía un plásmido coexpresando las secuencias de α y β-globina. La presencia de estas proteínas fue analizada por Western blot. Las globinas recombinantes tenían un peso molecular similar a las nativas indicando la ruptura del péptido de tránsito. La hemoglobina recombinante fue extraída de semillas y purificada por cromatografía. Esta hemoglobina fue estudiada en cuanto a propiedades funcionales como cinética de recombinación para monóxido de carbono encontrándose comportamientos similares a la hemoglobina humana. La producción de hemoglobina por este método posee las ventajas adicionales de eliminar la dependencia de sangre humana o bovina y las posibilidades de contaminación bacteriana y animal.

La producción de anticuerpos ha sido otro campo en el que se ha evaluado, por cierto con bastante éxito, la manipulación genética de plantas. En 1998 Verch y colaboradores (4), usando un vector basado en el virus mosaico del tabaco, lograron expresar en plantas anticuerpos monoclonales dirigidos contra un antígeno de cáncer de colon. Las cadenas pesadas y livianas fueron introducidas por separado y al coinfectar se logró el ensamblaje y funcionalidad del anticuerpo. También se ha reportado que anticuerpos del tipo IgA desarrollados en plantas tienen actividad contra *S. mutans*, el principal patógeno involucrado en el desarrollo de caries dental (5).

Se han realizado trabajos en la producción de vacunas para combatir infecciones que afectan gran cantidad de personas en el mundo, especialmente a los niños. *Escherichia coli* enterotóxica y *vibrio cholerae* causan diarrea aguda por colonización del intestino delgado y producción de enterotoxinas. La producción de vacunas eficaces debe estimular respuesta inmunitaria mucosa con producción de IgA secretora, un proceso que se logra mejor cuando el antígeno es presentado por vía oral. El gen de la enterotoxina de E. Coli enterotóxica fue introducido en plantas de tabaco y papa a través de *Agrobacterium tumefaciens* y se confirmó la presencia de enterotoxinas en estas plantas (6). Se alimentaron ratones por gavaje con extractos de hojas de tabaco transgénico y después de 30 días se evaluaron los títulos de anticuerpo contra la toxina, resultando similares a los títulos de los ratones a los que se administró la enterotoxina purificada de E. Coli.

La administración de papa transgénica produjo una respuesta de IgG e IgA específica contra la enterotoxina. Sin embargo la respuesta fue menor que cuando se administró la misma cantidad de toxina purificada. Esta trabajo muestra evidencias de la utilidad de las plantas para expresar y servir de vehículo de vacunas orales. Los mismos autores han realizado estudios expresando la proteína de cápside del virus Norwalk en plantas. La administración de estas plantas a ratones, provoca respuesta inmunitaria específica.

Con la finalidad de producir vacunas para niños se están realizando estudios en bananas, que expresarían antígenos "bioencapsulados" que serían consumidos y posteriormente liberados en el tracto gastrointestinal, produciendo una respuesta inmunitaria mucosa seguida inmediatamente por una respuesta humoral (7). Las bananas ofrecen múltiples ventajas incluyendo su alta disponibilidad y consumo en áreas tropicales y sub-tropicales donde usualmente las vacunas son mas necesarias, buen sabor y digestibilidad en infantes y no requieren cocción. La principal desventaja es que pueden transcurrir 2 a 3 años desde que se introduce el gen en las plantas, hasta que se obtiene el fruto transgénico.

Producción de alimentos

Desde el punto de vista de nutrición mundial, la búsqueda de mejoras en la producción de alimentos debe ser prioritaria. La población casi se duplicará en los próximos 45 años, lo cual implica que la producción de alimentos debería triplicarse. El área cultivable o de producción en el ámbito mundial podría variar muy poco. Por una parte tiende a disminuir debido a la urbanización y daño ambiental y por otra parte aunque es cierto que existen vastas zonas para la producción agrícola aún sin explotar, el costo de poner esas tierras a producir excede su valor, por lo menos en la actualidad.

Una de las utilidades de la industria genética es satisfacer la demanda creciente, aumentando la producción y calidad de los productos, probablemente disminuyendo la necesidad de grandes extensiones de terreno. Si se aumenta el contenido energético o el valor nutricional de los alimentos, disminuirá proporcionalmente la cantidad necesaria para alimentación, lo que es equivalente a aumentar la producción (8).

Se han realizado modificaciones en plantas que incluyen resistencia a insectos y herbicidas, resistencia a enfermedades y aumentar la calidad de alimento. A gran escala, estas mejoras se traducirían en disminución de costos, aumento de la disponibilidad de alimentos a nivel mundial y reducción del impacto sobre el ambiente. El control de insectos, hongos y otros organismos a través de plantas capaces de degradar toxinas producidas por estos organismos o la inserción de compuestos que no permitan a las aves o insectos acercarse a

la cosecha, disminuiría las pérdidas y reduciría costos. Con relación a mejorar el valor nutritivo de los alimentos, la utilización de modificaciones genéticas presenta- por lo menos en forma teórica- muchas ventajas. Sería posible introducir en un alimento de consumo masivo ciertos nutrientes en los que el alimento es deficiente, aumentar la disponibilidad de esos nutrientes, evitar la pérdida de nutrientes introduciendo algún elemento que lo retenga o lo haga mas absorbible, eliminar inhibidores de la absorción, etc.

La deficiencia de vitamina A, así como la de hierro y yodo constituyen importantes problemas de salud pública, que ocurren principalmente debido al consumo insuficiente o la baja biodisponibilidad de estos nutrientes. La fortificación de alimentos es una de las medidas mas efectivas para combatir la deficiencia. La inclusión de estos nutrientes o indirectamente de proteínas relacionadas con el metabolismo o la utilización de estos nutrientes, podría ser de gran ayuda en el mejoramiento del estado nutricional de la población.

Por ejemplo, el arroz es la principal fuente de energía de la mayor parte de la población mundial, especialmente en países en vías de desarrollo. Este cereal no contiene ni β-caroteno ni precursores C40 en su endospermo. Para mejorar el valor nutricional del arroz se ha tratado de introducir la maquinaria necesaria para que el tejido del endospermo del arroz sea capaz de producir β-caroteno y eventualmente, vitamina A. Burkhardt y colaboradores (9) encontraron en el arroz el precursor C20 necesario para la biosíntesis de carotenoides. La sintetasa de fitoene, que condensa 2 moléculas de geranil-geranil difosfato, es la primera de 4 enzimas necesarias para la síntesis de β-caroteno en plantas. Así, el arroz Japónica, variedad Taipei 309 fue transformado con el cDNA de la sintetasa de fitoene de narcisos, produciendo plantas transgénicas de arroz con la enzima de narcisos activa (medida por la acumulación de fitoene en endospermo de arroz). Los autores concluyen que es posible inducir la síntesis de vitamina A en un tejido vegetal no fotosintético carente de carotenoides, lo que tendría importantes implicaciones para disminuir la prevalencia de deficiencia de vitamina A.

Otro importante problema nutricional es la deficiencia de hierro y la anemia que acompaña sus últimos estadios. Este problema tiene alta prevalencia en grupos de edad en etapas de rápido crecimiento, cuando las demandas son mayores y está muy relacionado con la baja biodisponibilidad de las dietas consumidas en países en vías de desarrollo basadas en el consumo de cereales, tubérculos y leguminosas. En un artículo de Nature de este año, Goto y colaboradores (10) reportan sus experiencias sobre la fortificación de semillas de arroz con ferritina, como una manera de incrementar el contenido de hierro en este cereal.

La ferritina es la proteína de almacenamiento de hierro en los tejidos siendo capaz de almacenar hasta 4500 átomos de hierro. Esta proteína se encuentra virtualmente en todos los seres vivos y su conformación y capacidad de almacenar hierro, está bastante conservada entre los diferentes organismos.

Estos autores introdujeron el gen de ferritina de soya en plantas de arroz mediante transformación con *Agrobacterium tumefaciens*, bajo el control del promotor de glutelina (proteína de almacenamiento exclusivo de las semillas de arroz) de manera de acumular ferritina sólo en la semilla, reportando hasta 3 veces mayor concentración de hierro en semillas transgénicas que en las no transformadas.

En primer lugar aislaron el mRNA de soya y produjeron y amplificaron cDNA de ferritina utilizando primers específicos. Se hicieron 2 clonajes, el segundo de los cuales se realizó en un vector binario que contenía el promotor de glutelina. Este plásmido fue insertado en *Agrobacterium tumefaciens* por electroporación, la cual fue utilizada para infectar brotes de arroz. Estas plantas son cultivadas en el laboratorio y luego sembradas. Durante el proceso de cultivo y siembra, las plantas fueron analizadas para determinar la presencia del gen de ferritina de soya, encontrando por reacción en cadena de la polimerasa con transcriptasa reversa (RT-PCR) amplificación sólo en el caso de plantas transformadas. Además por Western Blot encontraron acumulación de ferritina exclusivamente en las semillas. En cuanto al contenido de hierro reportan incrementos importantes en semillas transformadas (en promedio 22.5 µg Fe/g peso seco en semillas transformadas y 11.2 µg Fe/g peso seco en semillas no transformadas). Además la determinación de hierro en otras partes de la planta demuestran que no hay diferencia en el contenido de hierro en plantas transformadas y no transformadas. Este hallazgo contrasta con el estudio realizado por Van Wuytswinkel y colaboradores (11) también en 1999, donde producen plantas de tabaco sobreexpresando el gen de ferritina de soya y encuentran alteraciones en el proceso de fotosíntesis que se evidencia por hojas amarillas, disminución del contenido de clorofila, desorganización estructural de cloroplastos. Por otra parte el incremento en la resistencia a metilviologeno, aporta evidencia adicional de la deficiencia de hierro en plantas sobreexpresando ferritina. La principal consecuencia de la acumulación de ferritina parece ser el aumento de hierro en las hojas y el incremento en la actividad de reductasa férrica (enzima crucial en la captación de hierro). Este comportamiento se explica debido al aumento en la capacidad de almacenamiento de hierro en las plantas transformadas, en las que se altera el metabolismo debido al excesivo secuestro de hierro, llevando a la planta a un "aparente" estado de deficiente que debe ser compensado activando los sistemas de captación de hierro.

Según Goto y colaboradores (10) es posible producir "arroz con ferritina" como una fuente de hierro para la dieta humana. El contenido de hierro de este arroz transgénico cubriría 30% a 50% del requerimiento diario de un adulto. Los resultados de este trabajo parecen indicar que estos métodos podrían ser un nuevo camino en la fortificación de alimentos para mejorar el estado nutricional de algunos grupos de población. Según la creciente cantidad de evidencia reportada

en la literatura, parece ahora una realidad muy factible la modificación de alimentos en gran escala con la posibilidad de incluir nutrientes importantes para el adecuado desarrollo humano. Es importante señalar que hay muchos elementos que analizar sobre todo en relación con riesgos potenciales, efectos secundarios, costos, beneficios, etc. En el caso específico de fortificación con hierro debe adicionalmente evaluarse la biodisponibilidad de esta fuente de hierro en humanos que, teóricamente, cubriría una buena parte del requerimiento diario. Como expresa Eleanor Lawrence en un editorial sobre este artículo (10) "quizás pasen algunos años antes que veamos este arroz con ferritina en nuestras mesas" pero lo que si es una realidad ahora es que este es un campo de investigación muy extenso que ofrece muchas posibilidades.

Actualmente existe preocupación acerca de los posibles riesgos de este tipo de manipulaciones. Uno de los puntos mas debatidos es la posibilidad de producir nuevos virus y por consiguiente nuevas enfermedades, mediante la manipulación genética. Esta es un área en la que se realiza una cantidad importante de investigación debido, entre otras cosas, al gran impacto económico que tiene la pérdida de cultivos por infecciones virales. Un método nuevo y poderoso para atacar este problema ha sido expresar ciertos segmentos virales (proteínas de cubierta, el gen incompleto de alguna otra proteína viral) en plantas, lo que confiere resistencia contra el virus correspondiente.

El problema que se plantea ahora (13), es la posibilidad de generar nuevos virus y enfermedades por medio de la recombinación genética. Un reporte de Greene y Allison (14) demuestra que ocurre recombinación cuando plantas transgénicas son inoculadas con un virus defectivo, y se recupera el virus completo. La preocupación es entonces si la recombinación genética podría producir nuevos virus peligrosos. La posibilidad de recombinación genética existe aún sin considerar plantas transgénicas, aunque se ha reportado que en los casos de infección viral mixta la producción de nuevos virus patógenos es muy rara. Aunque las condiciones en que se producen alimentos en muchos países proveen un buen ambiente para la interacción simultánea de varios virus, las nuevas enfermedades virales que se producen son usualmente debidas a variaciones menores de virus ya conocidos y no a virus nuevos de origen recombinante. La estabilidad estructural y funcional es importante y necesaria para el virus y los cambios son básicamente muy lentos.

En cuanto a las plantas transgénicas, aunque la respuesta no es aún definitiva, se cree que es poco probable que la recombinación entre RNA transgénico y genómico viral pueda ocurrir a frecuencias mayores que las que ocurren entre RNA genómico viral en infecciones naturales "convencionales" o múltiples, así como parece poco probable que puedan generarse y mantenerse nuevos virus viables mas patógenos o resistentes que los existentes, durante todo el ciclo de infección.

Otro tipo de patología que se ha incrementado en los últimos años, tanto en la severidad de los síntomas como en el espectro de susceptibilidad, son las alergias alimentarias. Por esta razón, la posibilidad de incluir alimentos transgénicos a nivel de poblaciones (13) ha causado gran preocupación en algunos sectores.

La inserción de un gen nuevo en una planta podría resultar en la expresión de nuevos alergenos o aumentar la expresión de los ya existentes. Si el DNA insertado se conoce bien, se podría esperar la expresión sólo de esa proteína y posiblemente el producto del gen marcador. Sin embargo, el sitio donde se inserta ese transgen en el genoma no está controlado y podría interferir con la expresión de otros genes y afectar numerosas características fenotípicas (15). Si la proteína a introducir es un alergeno, es muy probable que el organismo transgénico exprese la proteína con potencial alergénico. Una proteína de las nueces de Brasil (albúmina de almacenamiento 2S) fue insertada en soya para incrementar los niveles de aminoácidos azufrados y aumentar así el valor biológico de las proteínas utilizadas para producir alimentos para animales. Esta proteína es un potente alergeno (16) y provocó reacciones alérgicas idénticas tanto en la soya transgénica como en las nueces de Brasil (17). Así también se ha demostrado que la β-lactoglobulina expresada en E. coli tiene el mismo poder alergénico que la secretada en leche.

Se han sugerido (15,18) algunos aspectos que deben ser considerados antes de insertar una nueva proteína, entre los que se incluyen: conocer el origen del gen a insertar, caracterización exhaustiva de la proteína a producirse en el organismo transgénico, estudios en animales utilizando dicha proteína, análisis de estructura y comparación de secuencia con alergenos conocidos y predicción de estructuras alergénicas que podrían no estar accesibles antes del proceso digestivo. De esta manera podría disminuirse el riego de inclusión de una sustancia alergénica. Como puede desprenderse de lo aquí señalado, con los alimentos transgénicos y con la manipulación genética en general, las posibilidades son inmensas y los riesgos, hasta ahora, pocos. Queda sin embargo mucho que aprender y modificar para perfeccionar la metodología que podría permitir solucionar, entre otras cosas, gran parte de los problemas nutricionales de la población.

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