

Iron-Deficiency Anemia: Reexamining the Nature and Magnitude of the Public Health Problem

Iron Biology in Immune Function, Muscle Metabolism and Neuronal Functioning^{1,2}

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ABSTRACT The estimated prevalence of iron deficiency in the world suggests that there should be widespread negative consequences of this nutrient deficiency in both developed and developing countries. In considering the reality of these estimates, the Belmont Conference seeks to reconsider the accepted relationships of iron status to physiological, biochemical and neurological outcomes. This review focuses on the biological processes that we believe are the basis for alterations in the immune system, neural systems, and energy metabolism and exercise. The strength of evidence is considered in each of the domains and the large gaps in knowledge of basic biology or iron-dependent processes are identified. Iron is both an essential nutrient and a potential toxicant to cells; it requires a highly sophisticated and complex set of regulatory approaches to meet the demands of cells as well as prevent excess accumulation. It is hoped that this review of the more basic aspects of the biology of iron will set the stage for subsequent in-depth reviews of the relationship of iron to morbidity, mortality and functioning of iron-deficient individuals and populations. *J. Nutr.* 131: 568S–580S, 2001.

KEY WORDS: • iron deficiency • anemia • immune system • central nervous system exercise

Iron is both an essential nutrient and a potential toxicant to cells; as such, it requires a highly sophisticated and complex set of regulatory approaches to meet the demands of cells as well as prevent excess accumulation. A sufficient supply is essential for the functioning of many biochemical processes, including electron transfer reactions, gene regulation, binding and transport of oxygen, and regulation of cell growth and differentiation. This homeostasis involves the regulation of iron entry into the body, regulation of iron entry into cells, storage of iron in ferritin, incorporation into proteins and regulation of iron release from cells for transport to other cells and organs. This review will briefly remind all of us of the basic biology of iron (Webb 1992) and review the known biological aspects of the role of iron in immune system function (Hershko 1996), the biology of iron in neural functioning (Beard et al. 1993) and the role of iron in muscle function and energy metabolism (Beard and Dawson 1996). This review will not be comprehensive or exhaustive but will identify the key biological processes in each of the outcome domains identified as the focus of this conference (Fig. 1).

CHEMISTRY

Iron is a *d*-block transition element that can exist in oxidation states ranging from -2 to $+6$. In biological systems, these oxidation states are limited primarily to the ferrous ($+2$), ferric ($+3$) and ferryl ($+4$) states. The interconversion of iron oxidation states is not only a mechanism whereby iron participates in electron transfer but also a mechanism whereby iron can reversibly bind ligands. Iron can bind to many ligands by virtue of its unoccupied *d* orbitals. The preferred biological ligands for iron are oxygen, nitrogen and sulfur atoms. The electronic spin state and biological redox potential (from $+1000$ mV for some heme proteins to -550 mV for some bacterial ferredoxins) of iron can change according to the ligand to which it is bound. By exploiting the oxidation state, redox potential and electron spin state of iron, nature can precisely adjust iron's chemical reactivity. Thus iron is particularly suited to participate in a large number of useful biochemical reactions (Webb 1992). The general classification of these reactions is oxygen transport and storage, electron transfer and substrate oxidation-reduction. It is important to note that the activity of many of these enzymes decreases during tissue iron deficiency. Only rarely, however, have direct connections between biochemical events and clinical manifestations been firmly established.

Four major classes of iron-containing proteins carry out these reactions in the mammalian system: 1) iron-containing nonenzymatic proteins (hemoglobin and myoglobin), 2) iron-sulfur enzymes, 3) heme-containing enzymes and 4) iron-containing enzymes that are noniron-sulfur, nonheme enzymes

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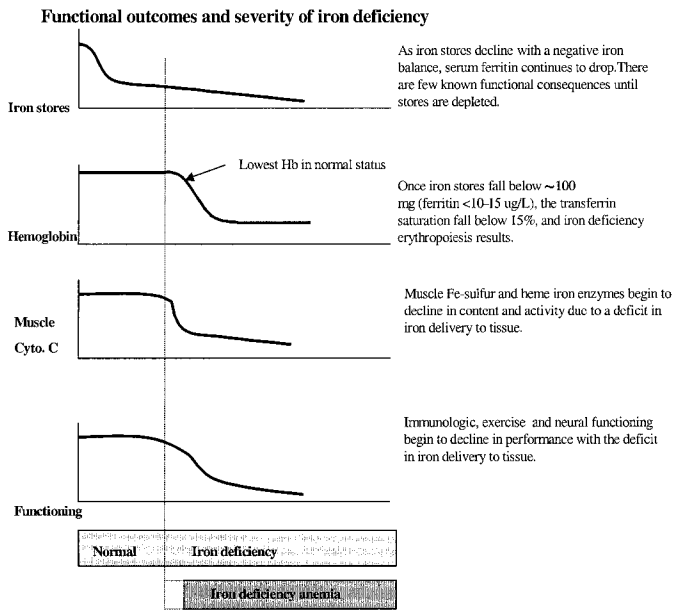


FIGURE 1 Diagram of changes in functional pools of iron at various stages of iron status.

(Fig. 2). In the principal oxygen transport nonenzymatic proteins, hemoglobin and myoglobin, iron functions as a critical ligand for the binding of dioxygen. In iron-sulfur enzymes, iron participates in single-electron transfer reactions primarily in energy metabolism. In the third category, iron is bound to various forms of heme and participates again in electron transfer reactions when associated with various cofactors (e.g., cytochrome P450 complexes). The final group of iron-containing enzymes is a catch-all grouping in which iron is not bound to a porphyrin ring structure or in iron-sulfur complexes.

Oxygen transport and storage. The movement of oxygen from the environment to terminal oxidases is one of the key functions of iron in which oxygen is bound to porphyrin ring

iron-containing molecules either as part of the prosthetic group of hemoglobin within red blood cells or as the facilitator of oxygen diffusion in tissue, myoglobin.

Hemoglobin is a tetrameric protein with two pairs of identical subunits ($\alpha 2, \beta 2$; MW 64,000). Each subunit has one prosthetic group, Fe-PP-IX, whose ferrous iron reversibly binds dioxygen. The synthesis of erythroid heme is controlled in part by the availability of iron to these maturing erythroblasts because iron regulates the initial rate-limiting step in heme biosynthesis by altering the stability of the specific [δ -amino-levulinic acid synthetase] mRNA. The four subunits are not covalently attached to each other but do react cooperatively with dioxygen with specific modulation by pH, pCO_2 , organic phosphates and temperature. These modulators of the affinity of hemoglobin for iron determine the efficiency of transport of oxygen from the alveoli capillary interface in the lung to the red cell-capillary-tissue interface in peripheral tissues. The allosteric effect of decreasing pH, the well-known Bohr effect, decreases the binding affinity of heme-Fe for dioxygen via protonation of His-146 on β chains and Val-1 on α chains in the presence of Cl^{-1} and CO_2 . CO_2 forms a Schiff base with the terminal amino acids of each chain and decreases dioxygen affinity. This favors the unloading of oxygen in tissues in which the pH is lower and pCO_2 is higher than in arterial blood. 2,3-Diphosphoglycerate is a product of a side pathway within erythrocytes and binds to a specific region of the β chain to decrease Hb- O_2 binding affinity. Homeostasis with respect to oxygen transport is evident in iron-deficient anemic individuals. There is usually a right shift of the dissociation curve with anemia, in which the blood content of hemoglobin is significantly reduced, and an increased cardiac output that is only partially compensatory. The increase in cardiac output is the result of an increase in both stroke volume and heart rate, with a resulting hypertrophy of the ventricular muscle wall that is concentric in nature (Mederios and Beard 1998). Physiological conditions that increase the demand for oxygen transport such as high physical exertion rates (discussed later in this review and by others at this meeting) may exist, with a resulting decreased maximal aerobic capacity because of limi-

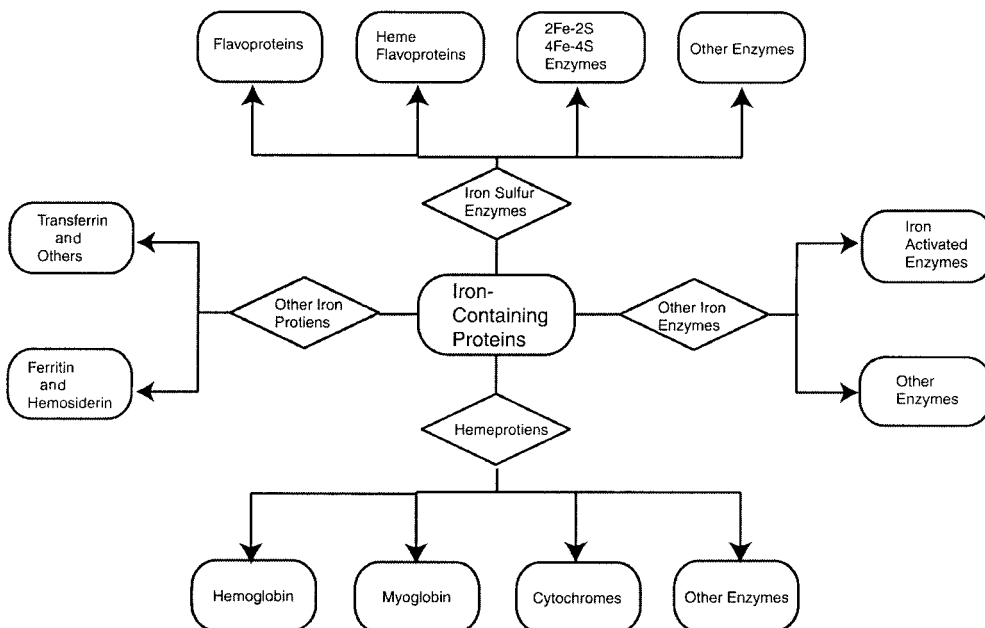


FIGURE 2 Classification of major iron-containing proteins.

TABLE 1

Enzyme activity in muscle from iron-deficient rats expressed as a percentage of control activity^{1,2}

| Tissue variable | Enzyme activity |
|---------------------------------------|-----------------|
| | % |
| Mitochondrial pyruvate-malate oxidase | 30 ± 3 |
| Mitochondrial succinate oxidase | 29 ± 5 |
| Mitochondrial cytochrome oxidase | 66 ± 9 |
| Mitochondria content in muscle | 72 ± 8 |
| Muscle cytochrome oxidase activity | 48 ± 7 |

¹ Values are means ± SEM. * $P < 0.01$, significantly different from control.

² Data derived from Davies et al. (1982).

tations in oxygen transport ability. It is uncommon, however, to find significant arterial desaturation unless very high rates of oxygen transport are required.

Myoglobin is the single-chain hemoprotein in cytoplasm (MW 17,000) that increases the rate of diffusion of dioxygen from capillary red cells to cytoplasm and mitochondria. The concentration of myoglobin in skeletal muscle is drastically reduced (40–60%) in tissue iron deficiency, thus limiting the rate of diffusion of dioxygen from erythrocytes to mitochondria (Beaton et al. 1989). The sensitivity of myoglobin concentration in other tissues to iron status has not been established.

Electron transport. The second group of iron-containing proteins comprises heme enzymes that use Fe within the porphyrin ring structure and are usually coupled with other enzymes in integrated electron transport processes. The overall scheme of the electron transport chain and the forms of iron that participate in electron transport are summarized elsewhere (Beard and Dawson 1996). The family of cytochromes contains heme as the active site, with the Fe-porphyrin ring functioning to reduce ferrous iron to ferric iron with the acceptance of electrons.

The third group of iron-containing proteins, the iron sulfur enzymes, also acts as electron carriers via the action of iron bound to either 2 or 4 sulfur atoms and cysteine side chains. The 40 different proteins that constitute the respiratory chain contain 6 different heme proteins, 6 iron sulfur centers, 2 copper centers, as well as ubiquinone to connect NADH to oxygen. There are numerous examples of these proteins and enzymes, which are summarized in Table 1. Although the sensitivity of many of these enzymes to iron depletion has been observed in one study or another, the systematic evaluation of tissue sensitivity for this modest list is lacking.

MODEL OF INTERORGAN IRON EXCHANGE

The dynamics of iron movement from dietary intake to interorgan distribution are well described by scientists in the fields of clinical science, nutrition and iron biology (Baynes and Bothwell 1990, Dallman 1982 and 1986, Finch and Heubers 1982). The regulation of iron movement across the enterocyte optimizes iron assimilation into the body when an individual is iron depleted and limits iron absorption from body iron status to the enterocyte remains unknown. A prolonged negative iron balance or an acute rapid blood loss both ultimately lead to a depletion of the storage iron pool, which can contain as much as 2–3 g iron (Fig. 3). The storage iron bound to tissue ferritin is used to meet the daily require-

ments not provided by the diet. Nearly all cells contain ferritin, although the bulk of the body's storage iron pool is in liver and spleen (Bothwell et al. 1979). Iron mobilized from tissue iron is transported by the iron transporter, transferrin. This plasma protein is normally only 25–50% saturated with iron bound to its two identical binding sites (Princiotta and Zapolski 1976). A negative iron balance will eventually lead to the depletion of the storage iron pools, with a resulting decline in the transferrin saturation to <15%, and less than adequate iron is available for delivery to essential body iron proteins (Bothwell et al. 1979).

The commonly accepted primary route of iron uptake into cells is mediated by the transferrin receptor on the cell surface. Newly described proteins such as divalent metal transporter-1 and stimulator of iron transport are now known to play a role in iron's movement from extracellular to intracellular spaces (Ponka et al. 1998). The regulation of this transferrin receptor population is influenced by the cellular iron status and its interaction with iron response elements (Casey et al. 1988). That is, as the cellular pool of low-molecular-weight iron decreases, there is an up-regulation of iron intake into cells and a down-regulation of the synthesis of the iron storage proteins. This influence is exerted via the existence of iron regulatory proteins in the nuclear fraction and their role in gene transcription and translation (Casey et al. 1988, Ponka et al. 1998, Rao et al. 1986).

Iron turnover and redistribution. Iron turnover is mediated primarily by destruction of senescent erythrocytes by the reticuloendothelial system (Finch et al. 1970). For example, in a 70-kg individual with a normal iron status, ~35 mg/d of iron is turned over in the plasma (Finch et al. 1970). Erythrocytes, which contain ~80% of the body's functional iron, have a mean functional lifetime of 120 d in humans. About 85% of the iron derived from hemoglobin degradation is rereleased to the body in the form of iron bound to transferrin or ferritin. Each day, 0.66% of the body's total iron content is recycled in this manner (Finch et al. 1970). Smaller contributions are made to plasma iron turnover by the degradation of myoglobin and iron-containing enzymes.

Storage and mobilization of iron. The concentration of iron in the body is ~30–40 mg/kg body. However, that concentration varies as a function of the age and gender of the individual and the specific tissues and organs being examined. About 85–90% of nonstorage iron is found in the erythroid mass (Bothwell et al. 1979). The storage iron concentration in

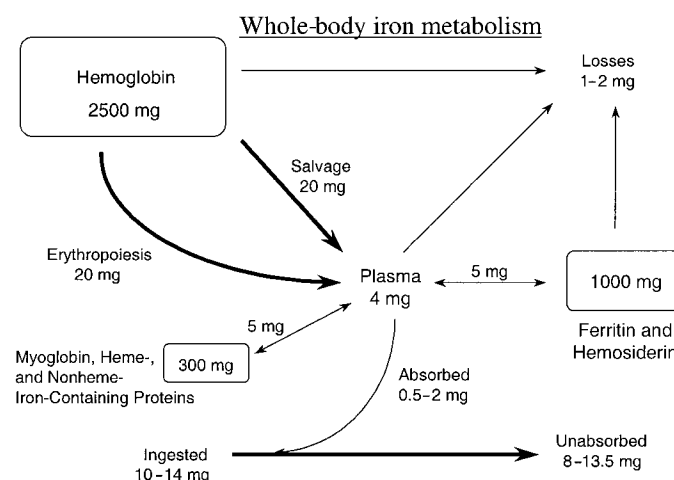


FIGURE 3 Iron distribution in the body and quantitative exchange of iron between body pools (Finch et al. 1970).

the body varies from 0 to 15 mg/kg body, depending on the gender and the iron status of the individual. The distribution of this stored iron is not uniform because the liver contains ~60% of the ferritin in the body. The remaining 40% is found in muscle tissues and cells of the reticuloendothelial system. Normally, 95% of the stored iron in liver tissue is found in hepatocytes as ferritin. Hemosiderin constitutes the remaining 5% and is found predominately in Kupffer cell lysosomal remnants. However, during iron overload, the mass of hemosiderin in the liver accumulates at 10 times the rate of ferritin (Green et al. 1968).

The overall structure of ferritin is conserved among higher eukaryotes and, in humans, is composed of 24 polypeptide subunits. At least two distinct isoforms of the polypeptide subunits exist, and combinations of these subunits allow for considerable heterogeneity in the structure of the full protein. The isoform designated H ferritin is a 22-kDa protein composed of 182 amino acids. The L isoform is a 20-kDa protein containing 174 amino acids. The subunit composition of ferritin seems to be tissue specific. Theoretically, up to 4500 ferric iron atoms can be stored in ferritin (Fishbach and Andreegg 1965). In vivo, ferritin is normally 20% saturated (800 of 4500 iron sites occupied) with a variable ratio of H to L subunits (Crichton and Ward 1992). The structure and composition of the mineralized core is analogous to a polymer of ferrihydrite ($5\text{Fe}_2\text{O}_3 \cdot 9\text{H}_2\text{O}$) with a variable amount of phosphate (Fishbach and Andreegg 1965). The H-chain possesses a distinct ferroxidase site, which leads to the current hypothesis that high H-L-ratio ferritin functions primarily to quickly move iron into and out of the core (Levi et al. 1988). The L-chain of ferritin lacks this ferroxidase site, and L-chain-predominant ferritin is viewed as a longer-term storage pool of iron (Casey et al. 1988, Levis et al. 1989). Pertinent to this discussion of the role of ferritin in the storage of iron is the proposed role of H-ferritin in growth and development and perhaps a role in nuclear regulation because H-ferritin receptors have been identified in several tissues and characterized in the nucleus of neurons (Hulet et al. 1999).

Iron losses. The low solubility of iron precludes excretion as a major mechanism of maintaining iron homeostasis. Thus, in contrast to most other trace minerals whose homeostasis is maintained by excretion, the primary mechanism of maintaining whole-body iron homeostasis is to regulate the amount of iron absorbed so that it approximates iron losses (Hallberg and Hulthen 2000). Iron losses can vary considerably with the gender of the individual and pathologies that have blood loss as a significant component. In male humans, total iron losses from the body have been calculated to be ~0.8–0.9 mg/d. For premenopausal female humans, this loss is slightly higher. The predominant route of loss is from the gastrointestinal tract and amounts to 0.6 mg/d in adult males (Finch et al. 1970). Fecal iron losses result from shed enterocytes, extravasated red blood cells and biliary heme breakdown products that are poorly absorbed. Urogenital and integumental iron losses have been estimated to be > 0.1 and 0.3 mg/d, respectively, in adult males (Green et al. 1968). Menstrual iron loss, estimated from an average blood loss of 33 mL/mo, equals 1.5 mg/d but may be as high as 2.1 mg/d (Cole et al. 1971). Oral contraceptives reduce this loss (Cole et al. 1971, Frassinelli-Gunderson et al. 1985), and intrauterine devices increase it (Cole et al. 1971, Guillebaud et al. 1979, Kivijarvi et al. 1986).

GENERAL CLINICAL MANIFESTATIONS

The overt physical manifestations of iron deficiency include the generic symptoms of anemia, which are tiredness, lassitude

TABLE 2

Symptomatology of iron deficiency

| |
|--|
| Anemia |
| Impaired thermoregulation |
| Impaired immune function |
| Impaired mental function |
| Impaired physical performance |
| Glossitis |
| Angular stomatitis |
| Koilonychia |
| Pica |
| Complications of pregnancy |
| Increased absorption of lead and cadmium |
| Altered drug metabolism |
| Increased insulin sensitivity |
| Blue sclera |
| Fatigue |

and general feelings of lack of energy (Table 2). Clinical manifestations of iron deficiency are glossitis, angular stomatitis, koilonychia (spoon nails), blue sclera, esophageal webbing (Plummer-Vinson syndrome) and microcytic hypochromic anemia. Behavioral disturbances such as pica, which is characterized by abnormal consumption of nonfood items such as dirt (geophagia) and ice (pagophagia), are often present in iron deficiency but clear biological explanations for these abnormalities are lacking. More recently, restless legs syndrome has been described as being causally related to iron-deficiency anemia (Earley et al. 2000b). This aphasic involuntary muscle contraction appears related to altered movement of iron to and within motor-control centers in the brain and is treatable in most cases with either iron or levodopa. Neuromaturational delays have been described by many research groups and will be discussed in detail in a later section and elsewhere in this supplement. The physiological manifestations of iron deficiency have also been noted in immune function, thermoregulatory performance, energy metabolism, and exercise or work performance (Beard et al. 1993, Lozoff 1988, Pollitt 1997). The current understanding of the iron biology underlying deficits in the immune system, skeletal myocyte and neural system will be discussed in the remaining three sections of this review.

Because nearly all of the functional consequences of iron deficiency are strongly related to the severity of anemia, the challenge of separating oxygen transport events from tissue iron deficits still looms large. However, this is largely an academic question because tissue iron deficits occur simultaneously with deficits in oxygen transport in naturally occurring iron-deficiency anemia. Good examples are the 50% decreases in muscle myoglobin content, cytochrome oxidase activity and electron transport capacity in skeletal muscle in subjects with iron deficiency, concurrent with a 50% decreased oxygen transport capacity due to anemia (Davies et al. 1982). Thus, although it is convenient at times to categorize individuals as iron-deficient anemic vs. iron-deficient nonanemic, this is not biological reality. Instead, it is more accurate to consider individuals along a continuum of iron nutriture with different functional consequences arising at different stages of severity.

IMPAIRED IMMUNE FUNCTION

Although most pathogens require iron and other micronutrients and have evolved sophisticated strategies for acquiring these micronutrients, iron is also required by the host for mounting an effective immune response. In a conceptual

model of nutritional immunity, the host must effectively sequester iron from pathogens and simultaneously provide a supply of iron that is not limiting to its immune system (Hershko 1996). There is new evidence that unicellular organisms and larger organisms, such as humans, share a common lineage of metal transporters (Fishbane 1999). These divalent metal transporters have been identified and cloned in both bacteria and humans and are used to internalize iron from extracellular spaces. This suggests that transport of iron is key to the survival of many pathogens, as well as that of the host organism. Natural resistance-associated macrophage proteins (NRAMP 1,2) are now known to be able to transport iron, zinc, copper, manganese and other divalent metals from endosomal vesicles into the cytoplasmic space. NRAMP 2 was recently renamed divalent metal transporter-1 or divalent cation transporter-1, reflecting its functionality as a proton-divalent metal countertransport protein (Ponka et al. 1998). Bacterial virulence is associated with the genes that code for iron acquisition in both *Escherichia coli* and *Vibrio* (Fishbane 1999, Ike et al. 1992). Thus, the acquisition of iron from biological fluids by siderophores secreted by bacteria is one of their routes of obtaining this essential nutrient. In vitro studies show that the provision of iron in rodents increases the pathogenicity of a number of bacteria (Sussman 1974). Data showing that this is also the case for humans are far less convincing and are reviewed in detail elsewhere in this supplement. An often-quoted study of Murray et al. (1978) examined Somali nomads with iron-deficiency anemia. Oral iron therapy led to a 12-fold increase in infections compared with no therapy. Replication of such a powerful effect of iron status has not been seen in other studies (Damsdaran et al. 1979).

Immunity during iron deficiency. Experimental and clinical data suggest that there is an increased risk of infection during iron deficiency, although a small number of reports indicate otherwise. Hershko (1996) urges caution in the interpretation of many studies because the confounding issues of poverty, generalized malnutrition and multimicronutrient deficiencies are often present in those studies. The molecular and cellular defects responsible for immune deficiency are complex because almost every effector of the immune response is limited in number or action by experimental iron deficiency. As mentioned previously, iron is essential for proper cell differentiation and cell growth. In addition, iron is a critical component of peroxide-generating enzymes and nitrous oxide-generating enzymes that are critical for the proper enzymatic functioning of immune cells. Finally, iron is likely involved in the regulation of cytokine production and mechanism of action through its influence on second-messenger systems (Hershko 1996). In one of few studies of role of iron nutrition in the development of the immune system, a delay was noted in the development of cell-mediated immunity (Kochanowski and Sherman 1985).

In adult animals or humans with intact immune systems, nonspecific immunity is affected by iron deficiency in several ways. Macrophage phagocytosis is generally unaffected by iron deficiency, but bactericidal activity of these macrophages is attenuated (Hallquist et al. 1992). Neutrophils have a reduced activity of the iron-containing enzyme, myeloperoxidase, which produces reactive oxygen intermediates responsible for intracellular killing of pathogens (Spear and Sherman 1992). There is a decrease in both T-lymphocyte number and T-lymphocyte blastogenesis and mitogenesis in iron deficiency in response to a number of different mitogens. This alteration is largely correctable with iron repletion (Kuvibidila et al. 1999, Spear and Sherman 1992). Recent studies of T lymphocytes in iron deficiency noted that protein kinase C activity and trans-

location of both splenic and purified T cells were altered by iron deficiency (Kuvibidila et al. 1999). On the other hand, others have found a normal T-lymphocyte proliferative response to mitogens (Canonne-Hergaux et al. 1999).

Humoral immunity appears to be less affected by iron deficiency than is cellular immunity. In iron-deficient humans, antibody production in response to immunization with most antigens is preserved (Hallquist et al. 1992, Spear and Sherman 1992).

Mediators of immune functioning. There are several possible mechanisms that could explain the effects of iron deficiency on the immune system. DNA synthesis, initiated by the iron-containing enzyme ribonucleotide reductase, is a rate-limiting factor in cellular replication and may be limited by iron deficiency. Control of differentiation of cells is influenced by the available iron and iron transport into cells via the transferrin receptor. Galan and colleagues (1992) reported a reduction in interleukin-2 production by activated lymphocytes in iron-deficient subjects. The release of interleukin-2 is fundamental to communication between lymphocyte subsets and natural killer cells but it does not appear to be the only cytokine that is altered by iron status (Sussman 1974).

Acute-phase reactants. Tumor necrosis factor, interleukin-1 and interferon- δ all work as effectors of iron movement. These cytokines operate in a coordinated fashion to reduce the size of the intracellular labile iron pool by reducing the amount of transferrin receptor on the cell surface, increasing the synthesis of ferritin for iron storage and activating nitric oxide systems (Damsdaran et al. 1979, Fishbane 1999, Hallquist et al. 1992, Ike et al. 1992, Kochanowski and Sherman 1985, Murray et al. 1978, Sussman 1974). Regulation of gene transcription is a likely mechanism. It is less apparent that the iron status of the individual can modify the acute-phase response system (i.e., does the ability to mount an acute-phase response depend in part on iron nutritional status?).

Nonetheless, there is a well-known decrease in the plasma iron concentration as well as an increase in the plasma ferritin concentration. Careful studies of the iron content of this newly released ferritin are not well established; thus the role of plasma ferritin in the sequestration of plasma iron remains uncertain. Sequestration of iron seems to be an important part of the host response to infection. Administration of a potent iron chelator, deferoxamine, to humans was examined to determine the potential antimalaria effect (Byrd and Horwitz 1989, Fahmay and Young 1993, Konijn and Hershko 1977, Lane et al. 1991).

MENTAL FUNCTION DURING IRON DEFICIENCY

Acquisition and location of iron. The brain obtains iron primarily via transferrin receptors expressed on endothelial cells on the brain microvasculature (Connor and Benkovic 1992, Fishman et al. 1987). The regulation of iron movement across this barrier is not well understood, although regulation in response to organ iron status clearly occurs. That is, the rate of iron uptake into the brain is increased when the iron status of the subject is low and is decreased when the iron status is higher (Taylor et al. 1991). In addition, the process is highly selective and not reflective of overall blood-brain permeability (Crowe and Morgan 1992, Morris et al. 1992). The uptake of iron is reported to be homogeneous and to be followed by redistribution to the basal ganglia, although there are other possibilities that depend on local regulation of uptake (Dwork et al. 1988). The choroid plexus is a rich source of transferrin mRNA, and transferrin secreted by this organ presumably is

used for the distribution of iron to glia and neurons for use or storage. It is important to remember that plasma transferrin can move across the blood-brain barrier and become part of the circulating pool of transferrin in cerebrospinal fluid. The adaptive regulation of transferrin synthesis and action in response to iron-deficiency anemia in the brain is unknown.

Regions of the brain rich in iron in adulthood, i.e., the substantia nigra, globus pallidus, nucleus accumbens, are not the regions that have a high iron content in early life. In addition, they are far less affected by dietary iron deficiency than are other regions such as the cortex or the striatum that have less iron content (Erikson et al. 1997). This iron is located primarily in microglia and oligodendrocytes and functions in numerous metabolic activities (Beard et al. 1993, Epstein and Connor 1999, Hill et al. 1985, Mash et al. 1990). 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 cerebellar nuclei particularly rich in iron (Aoki et al. 1989, Benkovic and Connor 1993). Recent studies from our laboratory and those of associates showed that iron accumulation in different brain regions is a function of the stage of brain development occurring at the time of the investigation (Fig. 4A, B) (Pinero et al. 2000). For example, when brain iron distribution is studied in a rodent model of lactational iron deficiency, the pattern of iron loss that emerges is entirely different from that for the effects of iron deficiency instituted during the postweaning period.

The different regional needs for iron in the brain during different stages of neurodevelopment could thus impart a differential sensitivity of brain regions to nutritional deprivation of iron (Erikson et al. 1997, Pinero et al. 2000). For example, in studies conducted in rodents during the mid- and late-lactational periods (equivalent to humans between 6 and 12 mo of life), there was a very significant 25% drop in cortex, striatum and hind-brain iron content with a short period of feeding a low iron diet. In contrast, there was only a 5% drop in thalamus iron content. During postweaning iron deficiency, there are comparable 20–30% declines in cortex, striatum and cerebellum, but the thalamus also becomes sensitive to dietary iron deficiency and has a 20% drop in iron concentration. These studies demonstrate that the effect of iron deficiency on brain iron content depends on the timing of the nutritional insult.

Iron and transferrin levels have been reported to be high in cerebrospinal fluid, especially in perinatal brains (Erikson et al. 1997). The actual levels of iron, however, are poorly described in conditions of iron overload and iron deficiency and during active growth and development. Atomic absorption spectrophotometry reveals iron concentrations of ~15–25 $\mu\text{g/L}$ in humans and monkeys and 5–20 $\mu\text{g/L}$ in mice (Bradbury 1997). These concentrations are ~5- to 10-fold lower than the corresponding plasma concentrations. Some unpublished data from our laboratory indicate that chronic diseases and pathologies (hepatitis, liver failure, diabetes) may alter these concentrations dramatically. In patients with restless-legs syndrome, the ratio of plasma iron to cerebrospinal fluid iron is abnormal, suggesting that the movement of iron across the blood-brain barrier is perturbed in these individuals (Earley et al. 2000a). The normal circulating level of transferrin in cerebrospinal fluid is also poorly described, although some reviews suggest that the total iron-binding capacity is barely even with the circulating iron concentrations, resulting in the apparent availability of free iron (Bradbury 1997). The role of the cerebrospinal fluid in the delivery of iron to various brain cells is not well understood (Dwork 1995, Malecki et al. 1998).

The brain is richer in the H subunit than in the L subunit

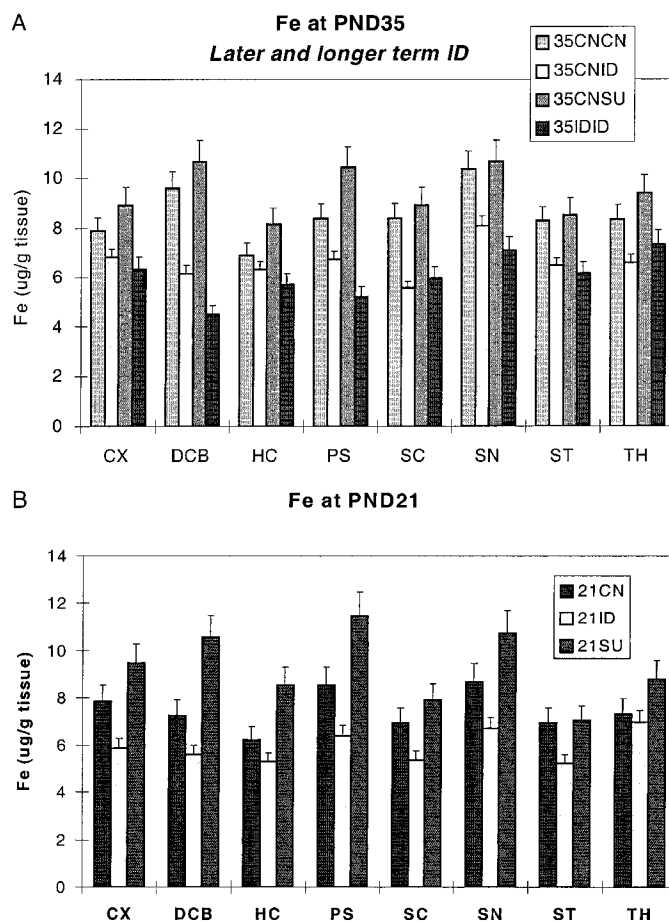


FIGURE 4 Regional brain iron concentrations (A) of rats made iron deficient from postnatal day (PND)10 to 35 (IDID), iron deficient from PND 21 to 35 (CNID), control from PND 10 to 35 (CNCN), or supplemented with 350 $\mu\text{g/kg}$ ferrous sulfate from PND 21 to 35 (CNSU) and (B) of rats made iron deficient from PND 10 to 21 (ID), control from PND 10 to 21 (CN), or supplemented with high levels of ferrous sulfate from PND 10 to 21 (SU). Values are means \pm SEM. Brain regions examined included the cortex (CX), the deep cerebellar nuclei (DCB), hippocampus (HC), pons (PS), substantia nigra (SN), striatum (ST) and the thalamus (TH). The distribution of iron was significantly different across regions ($P < 0.001$) and differed as a function of dietary treatment. See Pinero et al. 2000 for details.

of ferritin, and its localization is specific to cell type (Connor and Benkovic 1992). In rats and humans, microglia and oligodendrocytes contain ferritin, whereas in mice astrocytes contain ferritin. Ferritin levels correlate with brain iron content; they are highest at birth and decline thereafter in newborn rats (Miller et al. 1996). Moreover, the concentration can be directly affected by the body iron burden (Chen et al. 1995b). Ferritin isoforms are heterogeneously distributed in brain, and not all regions of the brain seem to be equally sensitive to an alteration in body iron status (Erikson et al. 1997, Kivijarvi et al. 1986). Studies in postnatal iron deficiency involving ferritin ratios (H:L) in pig and rodent brain reveal a dramatic effect of iron deficiency and the expression of these two subunits of the ferritin molecule (Erikson et al. 1998, Han et al. 2000). The changes in the protein expression appear to be regulated by the iron-response-element system as described previously. The developmental roles of the two subunits relative to iron storage or use and detoxification are unknown, although accumulation of iron in certain brain

regions is believed to play a role in a number of neuropathologies.

Iron deficiency effects. Dallman and co-workers (Dallman et al. 1975, Dallman and Spirito 1977) demonstrated two decades ago that young rats deprived of iron in early postnatal life have significantly lower (27%) whole-brain iron content than do controls 28 d postnatally and were quite resistant to restoration of their normal complement of brain iron (still 20% lower) despite aggressive dietary repletion for 45 d. Although these studies were landmark investigations at the time, they were usually misinterpreted to indicate that brain iron content was very static and not at all sensitive to dietary iron deficiency. This concept of a protected organ persisted for nearly two decades, until it was demonstrated by us and others that the brain is quite sensitive to dietary iron depletion and repletion and uses a host of mechanisms to regulate iron flux homeostatically.

When animals are given low iron diets in postweaning life, there is a significant decline in brain iron content and a rapid repletion with refeeding (Chen et al. 1995b, Erikson et al. 1997). This is in contrast to neonatal or preweaning iron deficiency in which the effects appear irreversible (Dallman and Spirito 1977, Felt and Lozoff 1996). From 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 published direct proof of this. Preliminary magnetic resonance imaging data from several restless legs syndrome patients with iron-deficiency anemia have weighted T2 relaxation times consistent with depleted striatal and nigra iron contents (C. Earley, unpublished data, personal communication). The areas of the brain that are quite sensitive to iron depletion in early life often are located within dopaminergic regions of the brain (Chen et al. 1995a, Erikson et al. 1997). Hill (1988) argues that the areas of highest iron concentration in the adult are not identical to those brain regions in which dopaminergic neurons either originate or terminate, which is not in agreement with Youdim et al. (1989).

Iron is required for proper myelination of the spinal cord and white matter of cerebellar folds (Larkin and Rao 1990), and it is a cofactor for a number of enzymes involved in neurotransmitter synthesis, including tryptophan hydroxylase (serotonin) and tyrosine hydroxylase (norepinephrine and dopamine). Iron is also a cofactor for ribonucleotide reductase, the rate-limiting step in DNA synthesis.

The predominant cell type containing iron in the brains of mice, rats, monkeys, pigs and humans is the oligodendrocyte (Hill 1988). These cells are responsible for the production of myelin; hence alterations in the functioning of these cells are associated with hypomyelination. Oligodendrocytes are responsible for the synthesis of fatty acids (Mackler et al. 1979, McKay et al. 1983) and cholesterol for myelin; both of these metabolic processes require iron. When oligodendrocyte maturation is disrupted, as results from some gene mutations, iron accumulation is only ~50% of normal (Connor and Menzies 1990). In iron deficiency, oligodendrocytes appear immature (Erikson et al. 1997). The failure to deliver iron to these cells during particular periods of early brain development could be causally related to delayed motor maturation and perhaps behavioral alterations in young humans (Rocangliolo et al. 1996). These investigators demonstrated a slowed nerve conduction velocity during an auditory evoked potential test. The reversibility of this finding remains under investigation. Although there are no quantitative data showing that iron deficiency leads to a lesser number of oligodendrocytes, hypomyelination occurs as a result of postnatal iron deficiency (Larkin and Rao 1990, Wiesinger et al. 2000). In addition, iron defi-

ciency could block cholesterol biosynthesis in these cells because at least three isoforms of P450 (a protoheme monooxygenase) are found specifically in oligodendrocytes.

IRON AND NEUROTRANSMITTER SYSTEMS

The role of intraneuronal iron in metabolism is varied and involves the following: incorporation of iron into enzymes of oxidation-reduction or electron transport; synthesis and packaging of neurotransmitters; and uptake and degradation of the neurotransmitters into other iron-containing proteins that may directly or indirectly alter brain function through peroxide reduction, amino acid metabolism and fat desaturation, thus altering membrane functioning.

Oxidation-reduction. Iron-dependent electron transport alterations in the brain resulting from iron deficiency are sparsely documented. Mackler and colleagues (1979) demonstrated that cytochrome concentrations in mitochondria from brain of iron-deficient animals were not different from those of controls. In addition, oxidative phosphorylation in these mitochondria using pyruvate-malate, succinate or α -glycerol phosphate was unaffected by iron deficiency. In animals with similar severities of iron-deficiency anemia (hemoglobin <60 g/L), skeletal muscle oxidative capacity was reduced by 40–50% (Sourkes 1973). At least superficially, it does not appear that there is a massive decrease in oxidative metabolism in the iron-deficient brain. This must be viewed with caution, however, because the brain is one of the most oxidative organs of the body.

Synthesis and degradation. Iron is a cofactor for tyrosine hydroxylase, tryptophan hydroxylase, xanthine oxidase and ribonucleoside reductase (Youdim and Green 1978). Thus, nutritional iron deficiency would be expected to lead to decreased activities of these enzymes; however, this has not been observed consistently. When brain iron levels are reduced by as much as 40% with dietary restriction in postweanling rats, there was no change in the activity of tyrosine hydroxylase, tryptophan hydroxylase, monoamine oxidase, succinate hydroxylase or cytochrome c oxidase (Ashkenazi et al. 1982). Aminobutyric acid transaminase and glutamate decarboxylase activities decreased, but these observations have yet to be reproduced by others. Whole-brain concentrations of norepinephrine and dopamine were unaltered by iron deficiency (Ashkenazi et al. 1982) and responded equally well to priming doses of levodopa (50 mg/kg). Serotonin and 5-hydroxyindole acetic acid concentrations were reported to be decreased. The turnover of norepinephrine, dopamine and serotonin in brain homogenates was also unaffected by iron deficiency, but more sophisticated approaches have not been used in recent years (Felt and Lozoff 1996, Youdim and Green 1977, Youdim et al. 1989).

Monoamine oxidase and aldehyde dehydrogenase are critical in the catabolism of neurotransmitters in the dopaminergic, serotonergic and noradrenergic systems of the brain (Youdim et al. 1975 and 1980). However, Mackler et al. (1979) were unable to demonstrate any decreased activity in brain of severely iron-deficient rats. Youdim et al. (1980) also showed no effect of iron deficiency in whole-brain preparations, although heart monoamine oxidase activity was decreased by >50%.

γ -Aminobutyric acid. Hill (1988) noted great similarity in the brain iron distribution and brain regions that receive input from γ -aminobutyric acid (GABA). She argued that the regions that have the highest concentrations of iron, the globus pallidus, substantia nigra, ventral pallidus and the cerebellar nuclei, also are highly innervated by GABA-mediated

nerve tracts. Because GABA release will modulate the activity of dopaminergic neurons, this is an important point to be resolved. In an older study, Youdim and colleagues (1980) observed no effect of iron deficiency on the GABA receptor population, amount of GABA or production rates. Other investigators, however, report that iron deficiency in utero and postweaning is associated with significant decreases in glutamate decarboxylase, glutamate dehydrogenase and GABA transaminase activities (Li 1998, Taneja et al. 1986). These latter two enzymes are shunt enzymes responsible for the synthesis and degradation of GABA. Thus, although concentrations of GABA may not be changed, there are some indications that GABA metabolism is altered by iron deficiency. Little substantive biochemical work has been done on this interaction, but such studies would expand the range of possible neurotransmitter systems that are altered by iron-deficiency anemia. No distinction has been made between intra-uterine effects of iron deficiency and later effects that may occur during lactation and after weaning.

Dopamine. The dopaminergic system in the only neurotransmitter system in the central nervous system that has been consistently sensitive to experimental changes in iron status. As whole-brain iron content drops 15% below normal, biological and behavioral alterations occur that may result from changes in the dopaminergic system (Erikson et al. 1997 and 2000, Morse et al. 1999a, Nelson et al. 1997, Yehuda 1990, Youdim 1990). Youdim and colleagues measured affinities and densities for dopamine D₁ and D₂ receptors, serotonin, GABA, benzodiazepine, and α and β adrenergic and muscarinic-cholinergic receptors in brain regions after postweaning dietary iron deficiency. They observed an effect of severe postweaning iron deficiency only on the dopamine system. The more recent experiments demonstrate that striatum dopamine D₁ and D₂ receptor densities are significantly lower (25–35%) in postweaning iron-deficient rats and that the dopamine transporter is also significantly lower in density in several brain regions (Erikson et al. 1997, Pinero et al. 2000).

Recent *in vivo* animal data demonstrate that extracellular dopamine is elevated in striatum of postweaning iron-deficient rats and returns to normal levels when brain iron content and iron status return to normal (Chen et al. 1995a, Nelson et al. 1997). Pharmacologic experiments with cocaine, a dopamine transporter inhibitor, demonstrate both *in vivo* and *in vitro* deficits in dopamine transport from the intersynaptic space back into presynaptic neurons (Erikson et al. 2000). Attentional processing of environmental information is highly dependent on appropriate rates of dopamine clearance from the interstitial space, which suggests that iron status may affect 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. However, lesions in many other parts of the brain may also result in alterations in perception, memory and motivation; thus the specificity of the connection between striatal dopamine changes and impaired spatial memory, attentional deficits and avoidance behavior remains to be established.

Serotonin and norepinephrine. As mentioned earlier, tryptophan and tyrosine hydroxylase are iron-containing enzymes that are essential for the production of serotonin from tryptophan and norepinephrine and dopamine from tyrosine (Webb 1992, Youdim and Green 1977 and 1978). As part of their initial survey of neurotransmitter systems that may be affected by iron deficiency, Youdim and colleagues measured the concentrations of serotonin, norepinephrine and their primary metabolites, 5-hydroxy indole acetic acid and

normetanephrine (Youdim and Green 1977 and 1978). They observed no significant alterations in concentrations of these neurotransmitters or metabolites in striatum of rats. Other studies of monoamine oxidase activity in brain of iron-deficient rats showed no effect on this enzyme responsible for the degradation of the monoamines, although platelet monoamine oxidase activity was affected (Youdim et al. 1975). Our laboratory has also examined these two neurotransmitter systems with *in vivo* microdialysis and failed to observe consistent alterations in their concentrations in extracellular fluid in the brain (Chen et al. 1995a, Nelson et al. 1997). However, radioligand binding studies performed in inbred strains of mice demonstrated significantly lower densities of the serotonin transporter in striatum of iron-deficient mice (Morse et al. 1999a). This observation has some significance because the dopamine, norepinephrine and serotonin transporters have a high degree of homology. Our observations of a consistent decrease in dopamine transporter density in striatum of iron-deficient rats, in combination with the one study of serotonin transporter, suggest a more general role of iron in the removal of neurotransmitters from the synaptic cleft.

Developmental models. Irreversible alterations in brain iron content have been shown in animal studies by feeding rats low iron diets early in life before the completion of the brain organization and myelination and the establishment of the dopaminergic tracts (Dallman and Spirito 1977, Felt and Lozoff 1996). Felt and colleagues demonstrated behavioral changes that could be associated with the irreversible changes in brain iron content that occurred, but they did not determine distribution of brain iron. This is in contrast to the ability of postweaning or late-lactational iron-deficient rats to recover brain iron content and functioning (Erikson et al. 1997, Pinero et al. 2000). In those studies, the investigators demonstrated a prompt recovery of brain iron in nearly all brain regions with feeding of a high iron diet. These studies, in combination, suggest that important biological switches for the acquisition of brain iron in early development may be irreversibly altered. A significant caveat to the observations from these rodent studies is that much of the rodent brain maturation occurs postnatally. Peak myelination of the rodent brain is occurring between postnatal d 8 and 14, whereas in the human infant, this occurs between ages 8 and 15 mo. Thus, the timing of the nutritional studies relative to interspecies comparisons must be carefully considered. Felt and Lozoff (1996) developed a very nice paradigm for this comparison that has been used very successfully by our laboratory to produce appropriate periods of iron deficiency to mimic lactational and early postlactational nutritional stress (Erikson et al. 1997, Pinero et al. 2000). Thus, much of the early disconnect between data collected in animal models and observations in human infants has been avoided. The next several years will see the publication of a number of articles from active research groups using rodent and primate models of iron deficiency that will further delineate the biological underpinnings.

IMPAIRED PHYSICAL PERFORMANCE

Muscle metabolism and energy use. Lethargy, apathy and listlessness are frequently observed symptoms of severe iron-deficiency anemia and perhaps anemia in general. However, for some time, iron deficiency has been known to be associated with decreased physical activity (Baynes and Bothwell 1990, Dallman 1982 and 1986, Finch and Heubers 1982). The consistency of evaluation of this effect on exercise tolerance and work performance hinges on the definitions of iron-defi-

ciency anemia that have used by investigators. This has led to some ambiguity in the results. However, the mechanisms of these effects have been thoroughly investigated in rodent models, and clear distinctions between the effects of diminished oxygen transport and oxidative capacity of muscle have been established (Dallman 1986). When rats are fed various contents of iron in purified diets, it is straightforward to establish that muscle myoglobin and cytochrome c are affected to a proportionally similar degree as hemoglobin with decreased iron intakes in young growing animals. That is, a severely anemic rat with a 50% decrease in hemoglobin concentration also has ~50% lower myoglobin and cytochrome c concentrations. That decreased exercise capacity is related to diminished oxygen transport, diminished oxygen diffusion within the exercising tissue and decreased oxidative capacity of muscle (Maguire et al. 1982, McLane et al. 1981). Although the efficiency of oxygen extraction is improved by the hemoglobin-oxygen saturation curve shifts, $VO_{2\max}$ is still decreased 30–50% in both animals and humans.

The manifestations of depletion of essential body iron also have profound effects on skeletal muscle, with a significant decrease in mitochondrial iron-sulfur content (Maguire et al. 1982), mitochondrial cytochrome content (McKay et al. 1983, McLane et al. 1981, Willis et al. 1987) and total mitochondrial oxidative capacity (Davies et al. 1982 and 1984, McKay et al. 1983, Willis et al. 1987). Pyruvate and malate oxidase were decreased to 35% of normal in iron-deficient muscle and improved to 85% of normal after 10 d of iron treatment. 2-Oxoglutarate oxidase was decreased to 47% of normal and improved to 90%. In contrast, succinate oxidase was only 10% of normal in iron deficiency and improved to only 42% of normal after 10 d (Perkkio et al. 1985). The cytoplasmic enzymes, hexokinase and lactate dehydrogenase, were unaffected by iron status. The 50–90% decrease in both the iron-sulfur enzymes and in the heme-containing mitochondrial cytochromes is consistent with many observations over the past two decades (Dallman 1986, Willis et al. 1987). What seems to determine the amount of decline in activity with iron deprivation is the rate of turnover of that particular iron-containing protein in the time of cellular deprivation of iron. Although this makes sense conceptually, the direct testing of this hypothesis occurred in a cursory fashion.

Exercise performance at the time of severe iron-deficiency anemia was only 20% of normal in a brief, intense exercise protocol (Willis et al. 1987). Treatment with iron dextran corrected this exercise tolerance within 4 d, but mitochondrial oxidation using either malate-pyruvate or succinate as substrates was not improved. When α -glycerol phosphate was used as the substrate, however, a very significant improvement in the rate of oxidation was realized, suggesting that this is the rate-limited step in iron-dependent oxidation. Longer-term repletion led to the normalization of other enzyme activities (Willis et al. 1990). The interpretation of these data is consistent with rates of turnover of cellular enzymes that require iron and thus support the premise that rates of loss of enzymatic function in muscle are related to turnover of iron-containing enzymes within those cells. A study using ^{31}P nuclear magnetic resonance spectroscopy to examine the functional state of bioenergetics in iron-deficient and iron-replete rat gastrocnemius muscle at rest and during 10 min of contraction at 2 Hz demonstrated an effect of iron status (Thompson et al. 1993). Iron-deficient animals had a clear increase in phosphocreatine breakdown and a decrease in pH compared with controls and a slower recover of phosphocreatine and inorganic phosphate concentrations after exercise. During repletion for 2–7 d with iron dextran, there was no substantial

improvement in these indicators of muscle mitochondrial energetics. These authors concluded that “tissue factors” such as decreased mitochondrial enzyme activity, decreased number of mitochondria and altered morphology of the mitochondria might be responsible for these observations.

Anemia vs. tissue iron deficiency. Dallman and colleagues established the concept that anemia limits the capacity of the individual to deliver oxygen to exercising muscle, whereas tissue iron deficiency limits the capacity of the individual to perform oxidative metabolism (Dallman 1982 and 1986). This was accomplished by iron-repletion and exchange-transfusion studies and the observation of recovery of sprint performance compared with endurance performance (Fig. 5). There was a stronger correlation of sprint performance with anemia than with tissue cytochrome c content, whereas there was a strong correlation of tissue cytochrome c with recovery of endurance performance. Exchange-transfusion experiments in which the hemoglobin concentration of animals was manipulated by transfusion further demonstrated that endurance performance is almost entirely related to muscle metabolism and relatively independent of hemoglobin concentration down to 100 g/L.

Fuel homeostasis. Iron-containing enzymes in skeletal muscle and liver are altered in iron deficiency to promote an increased rate of lactate production in muscle and use by liver (Davies et al. 1982, Finch et al. 1979, Henderson et al. 1986, Ohira et al. 1986, Thompson et al. 1993). These animal studies clearly demonstrated that the rates of plasma disappearance of radioactively labeled glucose and lactate were both increased in iron-deficiency anemia. Even at rest, there were higher concentrations of glucose in the plasma of iron-deficient animals than controls. Additional studies demonstrated that lactate dehydrogenase is increased in activity in iron-deficient skeletal muscle and that isozyme adaptations occur to maximize this capacity for anaerobic metabolism (Ohira et al. 1986). In contrast, other more recent experiments suggest that a number of nonheme iron-sulfur proteins, especially aconitase, may be very susceptible to variations in cellular iron content (Willis et al. 1990). Dallman's laboratory demonstrated that this key enzyme in gluconeogenesis could be restored to full activity with 15 h of an iron injection in the rat model, with a rapid return of lactate and glucose concentrations toward normal. Rates of gluconeogenesis in iron-deficiency anemia are increased to improve the provision of blood

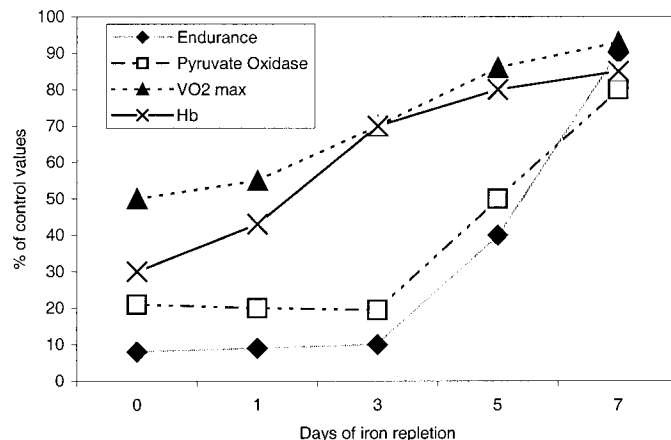


FIGURE 5 The relationship of iron repletion to hemoglobin response (Hb), maximal aerobic capacity ($VO_{2\max}$), muscle pyruvate oxidase activity and endurance capacity in rats made iron deficient post-weaning. Redrawn from Dallman 1982.

glucose (Klempa et al. 1989). The endocrine basis of the glucose cycling was examined in our laboratory. We demonstrated a dose-response relationship among hyperglycemia, hyperinsulinemia and severity of iron-deficiency anemia (Borel et al. 1993, Farrell et al. 1988). Hyperinsulinemic euglycemic glucose clamps were used to demonstrate an increased sensitivity to insulin in animals with hemoglobin concentration < 120 mg/L. This was also the threshold for a significant elevation in fasting blood glucose concentration. Interestingly, similar observations on fuel homeostasis alterations have not been reported in humans who have a similar proportional severity of anemia. It is important to recall that the catecholamines (epinephrine and norepinephrine) are key regulators of glucose production and that their concentrations in plasma are abnormal in iron-deficient anemic humans and rodents (Beard et al. 1988b, Dillman et al. 1980, Martinez-Torres et al. 1984). Taken together, these studies illustrate that tissue iron is associated with an increased reliance on glucose as a fuel for endurance performance at submaximal workloads, whereas hemoglobin-associated iron plays an important role in oxidative capacity and maximal aerobic work performance.

Human studies. Although it is the responsibility of others attending this workshop to address the human literature on the effects of iron deficiency on work performance, it is appropriate to return to one of the key research questions that still remain unresolved (Viteri and Torun 1974), i.e., is there a linear relationship of iron status to work performance or is there a curvilinear relationship with a plateau? This question is centered on the notion of a critical cut-off value for the severity of anemia above which there is no relationship and below which there is a strong relationship. Viteri and colleagues used a Harvard Step Test to demonstrate in a laboratory setting that performance was linearly and positively correlated with hemoglobin over the entire range normally seen in humans (Fig. 6A). In contrast, the data of Gardener and colleagues (Edgerton et al. 1981) clearly demonstrate that time to exhaustion for submaximal exercise has a curvilinear relationship to hemoglobin (Fig. 6B). The apparent conflict between these two data sets may be resolved if we are willing to accept the animal experiments of Finch and Dallman's research groups. That is, lower intensity endurance exercise is tightly correlated with tissue iron deficiency where a curvilinear relationship is expected, whereas a brief intense exercise (Harvard Step Test) should be more tightly correlated with severity of anemia (Davies et al. 1982, Finch et al. 1979). Arterial oxygen content, oxygen delivery bound to hemoglobin and cardiac output are all key determinants of the amount of work that exercising muscle can do. Gardner and Edgerton evaluated the physical work capacity and metabolic stress in iron-deficient workers on a tea farm in Sri Lanka (Edgerton et al. 1979). In one study, men and women with hemoglobin concentrations of 40–120 g/L were assigned to either an iron-treatment or a placebo group. Maximal exercise tolerance was dramatically reduced in the anemic subjects who also transported 15% less oxygen per pulse than did nonanemic subjects. Iron treatment restored cardiorespiratory and work performance variables.

A number of studies note that there is an elevation in lactate concentrations in iron-deficient subjects relative to control subjects (Celsing et al. 1986, Ekblom 1997). This is most easily interpreted as follows: the anemic subjects had a lower aerobic threshold relative to control subjects; thus, exercise protocols at a fixed level of high effort were more likely to put anemic subjects above the threshold. Thus, the proto-

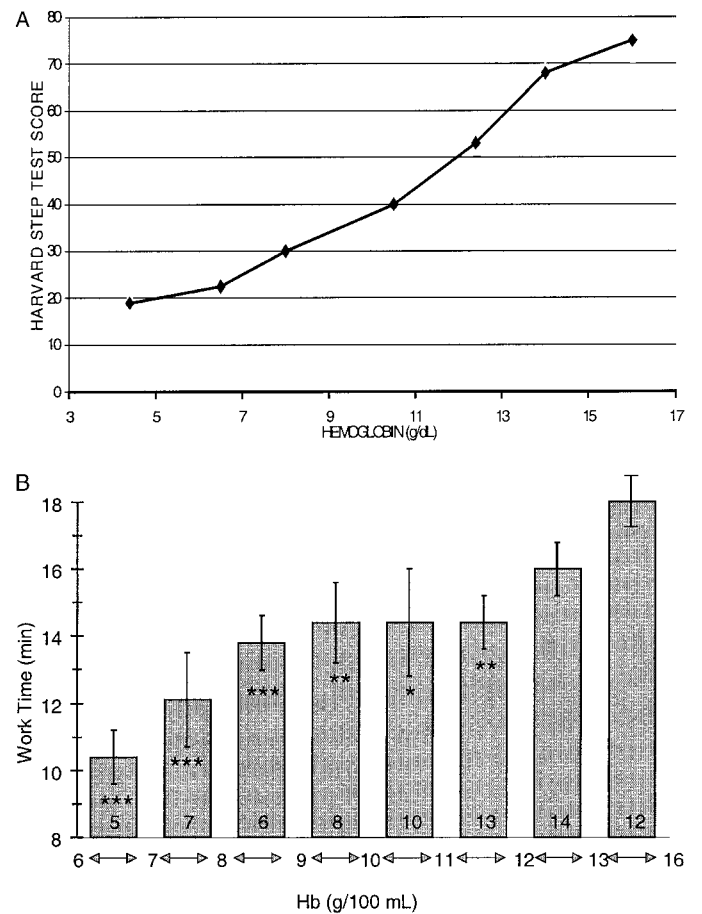


FIGURE 6 (A) Harvard Step Test score among a group of Guatemalan agricultural laborers. Impaired performance occurred even with mild anemia. Redrawn from Viteri and Torun 1974. (B) Work time in a group of Indonesia agricultural workers performing a submaximal exercise test as a function of severity of anemia. A clear plateau is achieved at moderate severity of anemia. Values are mean \pm SEM. Significant differences from the highest hemoglobin group are noted as follows: * $P < 0.05$; ** $P < 0.01$; and *** $P < 0.001$. Redrawn from Gardner et al. 1977.

cols that are used have a large effect on the conclusions regarding the role of iron in exercise performance.

SUMMARY AND CONCLUSIONS

This nonexhaustive review of the biological aspects of iron and immunity, brain function and exercise tolerance identifies major areas of ignorance in our knowledge base. The nature of the biological dose-response relationship between severity of anemia and functional outcome remains to be identified in most cases. Developmental dependence has largely been ignored except for the studies on neural development. There is a virtual absence of work on second-messenger systems that are the likely intermediaries of the effects of low iron status on immune cell functioning and fuel homeostasis. Animal model and cell culture studies continue to demonstrate new relationships between iron and cell biology. The application of these finding to humans remains a great challenge.

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DISCUSSION

Participants: Oppenheimer, Beard, Lozoff, Pelletier, Habicht, Pollitt, Brabin

Dr. Oppenheimer: How comfortable are we comparing myelination in the newborn rat with the newborn human?

Dr. Beard: The peak time of myelination in the rodents is between 8 and 14 d of postnatal life with peak at about 11–12 d. In the human, it is going to be somewhere between 8 and 20 mo, with the peak probably around 14–18 mo. In the human it is much more prolonged. In the rat it is compressed. We used the model on purpose to allow us to define the timing of these events.

Dr. Lozoff: It is also important to know that different systems myelinate at different rates. It is not just a question of the species but the function being looked at.

Dr. Pelletier: One can see why the Corey cycle might be a functional adaptation in iron deficiency, but why would one necessarily expect the increased glucose concentration instead of simply normal glucose concentrations?

Dr. Beard: Well, there is not an easy answer to that. It probably has to do with this insulin sensitivity issue—that this was tied into rates of glucose clearance. Why the set point changes, I do not know. Nobody that I know of is doing any work on glucose and insulin homeostasis and iron.

Dr. Oppenheimer: These were all reversible changes of glucose metabolism?

Dr. Beard: Yes, they are all reversible.

Dr. Habicht: Coming back to something you talked about

earlier; regarding neurology, these outcomes were reversible, such as myelination and so on. What degree of iron deficiency was there compared with what we would see in a human population?

Dr. Beard: The severity of iron deficiency is hemoglobin around 60–70 g/L and the question is one of reversibility. The animals that were always iron-deficient anemic do not do anything. They are like doorstoppers. They do not explore the environment. However, if we give those animals iron, in as little 2 wk, from d 21 to 35, they improve dramatically. So, they do have this capacity to improve, but you will notice that it is not the same as the controls. They do not reach the same level of performance as animals that were never iron deficient.

Dr. Lozoff: This is not necessarily a myelin problem.

Dr. Beard: Not necessarily myelin.

Dr. Habicht: The question I had was at what degree of deficiency do you see these effects?

Dr. Beard: One answer to your question is the study that Drs. Felt and Lozoff published a few years ago in which they had a much milder degree of iron deficiency than what we have. They showed a defect in the homing behavior of these animals. They had very mild levels of anemia—hemoglobin around 100–110 g/L. Some data in the animal literature show that even at mild degrees of iron deficiency anemia where there is no growth retardation, no other overt signs of abnormalities in behavior occur. What Barbara Felt did not have in those data are any of the fine-grained analysis that we have been able to do, but we have not yet gone back to look at the moderate anemia.

Dr. Pollitt: I have two points to make. One is that there does not have to be a one-to-one correspondence between the kinds of operations that you have described and cognitive or other types of behavioral manifestations in children. In other words, even if you are able to demonstrate that there is reduced conduction in the auditory system, that does not mean that there is actually a functional correspondence.

The second point is that it is unfortunate that the old work on activity and on muscle in animals and humans has only been related to work performance and not really to motor development as well. Those data could actually be related to other forms of development that could be extremely important for the child's adaptation.

Dr. Beard: I agree. You are absolutely right.

Dr. Pollitt: The amount of information that we now have on the functional consequences of iron deficiency is so broad that you would expect that development is going to be affected by very different kinds of pathways.

Dr. Beard: For the first time we are starting to get a clue that different parts of neural circuitry may be more sensitive to iron at different times. For example, the cerebellum seems to be quite sensitive during an early time period as well as a little bit later, whereas the thalamus is not sensitive at all during an early time but it is quite sensitive later. So, one could by virtue of knowing that piece of biology then start to look at the kinds of data you and Dr. Lozoff and others have collected and argue that if we have iron deficiency at that time, maybe this circuit is not developing. You are right. We need to have circuit-specific outcome variables.

Dr. Oppenheimer: Can you do intellectual tests on rats, such as maze learning and long-term memory?

Dr. Beard: Yes. You can do spatial learning and long-term memory kinds of things. It is all possible to do. Not much has been published on that yet.

Dr. Brabin: Can you tell me something about nonsomatic uterine muscle? The duration of labor—would it be prolonged? I was particularly interested in nonsomatic muscle because of the problems with uterine function in labor. These could be major problems in women who have a high prevalence for iron deficiency.

Dr. Beard: Yes, you would think that muscle contractility would be severely compromised in that situation.