Pathogens, Metabolic Adaptation, and Human Diseases—An Iron-Thrifty Genetic Model

Pathogens and famine have dictated the fate of humans throughout their evolution (Figure 1). Fighting pathogens and avoiding starvation have most likely represented the greatest challenges for humans since they diverged from their closest evolutionary relatives, the chimpanzees, some 10–13 million years ago. Central to our species' success in both of these challenges is iron, which is among the most abundant elements on Earth and an important micronutrient in human diet. Our bodies contain much more iron than any other essential trace element, such as copper (4–5 g Fe but only 80–100 mg Cu). Iron enables hemoglobin in erythrocytes and myoglobin in muscles to bind and deliver oxygen; iron is also used, for example, by mitochondria in the synthesis of high-energy compounds such as adenosine triphosphate. Iron is so critical for us that, once it is absorbed via the distal duodenum and enters the bloodstream, it can be transferred across cell membranes and reach any cell of the body with few obstacles.

From Single-Cell Organisms to Higher Animals: The Evolution of Iron Metabolism

Unfortunately for us, most human pathogens—bacteria, fungi, and protozoa—are devoid of iron. To acquire this element from the external environment and use it for growth and proliferation, these microbes evolved sophisticated molecular pathways for iron long before humans appeared in the evolutionary tree. In fact, the first living organisms to appear on Earth, some 4 billion years ago, formed in the iron-rich oceans that dominated the surface of the planet. Under the oxygen-free atmosphere that blanketed the world, before the advent of photosynthesis, single-celled Archaea, among the most ancient microorganisms, were producing energy from inorganic compounds using iron–sulfur clusters, the earliest catalytic cofactors on Earth.[1]

The chemistry of early life, in fact, was founded on the capacity of water-soluble ferrous iron (Fe^{2+}) to exchange electrons with ferric iron (Fe^{3+}). Yet some 2.3 billion years ago, a major catastrophe called the *Great Oxygenation Event* changed forever the fate of our planet and our own lives[2]: oxygen, largely produced by cyanobacteria—a phylum of bacteria quite different from Archaea, began to accumulate in the Earth's atmosphere. In this altered atmosphere, soluble Fe^{2+} was oxidized to insoluble Fe^{3+}, and hence the bioavailability of iron was lost. Moreover, because iron in the presence of oxygen catalyzes the generation of noxious reactive oxygen species that damage macromolecules like proteins and nucleic acids, it became a potentially toxic agent.

In this scenario, organisms evolved a series of iron-mobilizing proteins to take up iron from the environment and deliver it to tissues. Higher animals (those with a gastrointestinal system) began to express divalent metal transporter 1 (DMT-1), tasked with transferring iron across the apical surface into the cytosol of the enterocytes. A multimeric iron-binding protein called ferritin evolved to store iron in cells safely away from oxygen. A serum protein called transferrin evolved to securely transport insoluble Fe^{3+} in the bloodstream and deliver it to tissues, by binding to transferrin receptor 1. And, to move iron from the enterocytes into the bloodstream, nature invented a transmembrane channel called ferroportin (encoded by the *SLC40A1* gene). Ferroportin is among the earliest iron proteins to evolve; as the sole iron exporter in mammals, it is expressed not only by the iron-absorbing enterocytes but also by the iron-storing hepatocytes and iron-recycling macrophages.[3] Ferroportin is so important for us that its complete loss owing to homozygous inactivating mutations is incompatible with life, whereas its partial loss owing to heterozygous inactivating mutations causes an autosomal-dominant iron withholding syndrome known as ferroportin disease.[4] A functioning hepcidin–ferroportin axis is found in mammals and fish, whereas lack of *HAMP* suggests that iron homeostasis may be regulated by an alternative mechanism in birds.[5] In general, iron proteins are phylogenically highly conserved throughout different vertebrate species, in other chordate subphilum, such transferrin and DMT-1 in the tunicate *Ciona*, whereas ferritins are also found in early metazoa, plants, eubacteria, and Archaea.

But what about pathogens? The persistent flow of iron into the bloodstream sustained by ferroportin may have been a lifeline for humans and other animals, but it also represented a true bonanza for bacteria during infection. The virulence of infectious organisms depends on their ability to assimilate iron from their hosts, so bacterial pathogens evolved refined mechanisms to "steal" iron from human proteins.[6] An example for all is the long evolutionary battle[7] between primate transferrin and bacterial transferrin-binding protein (TbpA). Over millions of years, the "predatory" TbpA has co-evolved with its "prey," transferrin. When sequence modifications in transferrin precluded the bacterial protein from binding and carrying out its "iron piracy," the bacteria responded to this selective pressure by producing a mutated TbpA with renewed function.[7] There is also clinical evidence linking infection to the abundance of iron.[8] In humans with iron overload syndromes, infections with some bacteria and viruses can be more frequent and virulent, whereas iron-deficient individuals seem to be protected from some other infectious diseases (eg, malaria).

How did humans and other mammals cope with the bacterial piracy of

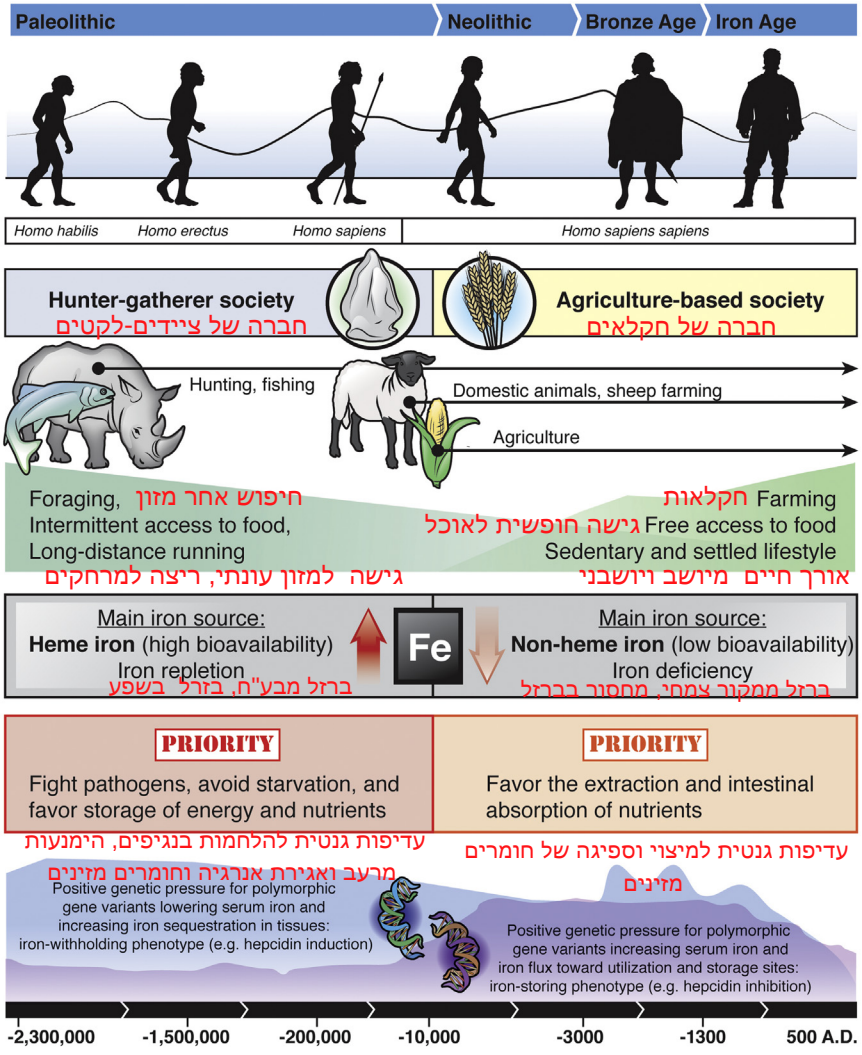

Figure 1. Pathogens and famine have dictated human priorities and altered the pattern of genetic selection during evolution. The change from the hunter–gatherer lifestyle of the Paleolithic period to the settled agrarian societies of the Neolithic period had profound effects on human nutrition and disease and favored the emergence of opposite genetic traits of iron metabolism.

iron during infection? Our innate immune system combats invading pathogens by expressing a family of antimicrobial peptides. One of these, called hepcidin, has the unique ability to shut down ferroportin and limit the export of iron into the bloodstream. Hepcidin (encoded by the *HAMP* gene) is a 25-amino acid peptide secreted by the liver in response to bacterial or viral infections. This peptide hormone is the natural ligand for ferroportin: when it binds ferroportin, the cells endocytose the complex and degrade it, preventing further iron efflux.[9] Unfortunately, the mechanism is so effective that, during chronic infections, the prolonged elevation of hepcidin levels may lead to iron-restricted erythropoiesis and anemia, a syndrome known for years as anemia of inflammation or anemia of chronic disease.[10]

Hepcidin is also sent into action when serum iron approaches toxic levels. Serum iron is monitored by hepatocytes via an iron-sensing membrane complex comprising bone morphogenetic proteins (BMP), BMP receptors and co-receptor (hemojuvelin), and ancillary proteins including the hereditary hemochromatosis protein HFE.[11] In the case of excess serum iron, this system triggers hepcidin production, which results in the degradation of ferroportin in enterocytes and restores normal levels. Conversely, when iron is scarce, hepcidin transcription is repressed so that intestinal iron absorption is maximized and marrow iron requirements are met.

Despite hepcidin's importance in iron homeostasis, total loss of this hormone is not fatal, but lack of hepcidin (or its key regulator, hemojuvelin) results in the unchecked release of iron from enterocytes and macrophages into the blood, causing massive iron overload in young people; this syndrome is called juvenile hemochromatosis. Although loss of hepcidin does not preclude an organism's ability to fight infection, lack of the hepcidin ancillary protein HFE at the hepatocyte plasma membrane (as occurs in homozygotes for the C282Y mutation) causes a more common but less severe form of hemochromatosis that affects Caucasian adults,[11] and seems to be associated with higher susceptibility to infections owing to siderophilic microorganisms, such as *Vibrio vulnificus* and *Yersinia enterocolica*.

Fighting Pathogens and Coping With Famine: How Humans Evolved the Iron-Withholding and Iron-Storing Phenotypes

During human evolution, a dramatic change in nutrition and lifestyle occurred with the advent of farming some 10,000 years ago (Figure 1). In what is called the Neolithic Revolution, the nomadic hunter–gatherers of the Paleolithic period gave way to agrarian societies. Before this change, Paleolithic men and women engaged in intense physical activity while hunting big game species, and ate mostly lean meats and foraged plant foods. Because meat provided large amounts of iron in a readily absorbable heme form, they were not iron deficient. The Neolithic diet, instead, offered less iron and in a poorly usable form, also because the cultivated cereals had antiabsorptive properties. Iron-deficiency anemia was common in these crowded agrarian communities. Thus, although access to food was less of a problem than during the Paleolithic age, iron shortage was a reality.

For the Paleolithic people, the main environmental stresses were infectious diseases and intermittent access to food. The settled Neolithic civilization was even more exposed to infectious diseases and parasites.[12] Parasites transmitted in the soil (eg, hookworms), which are extremely avid of iron, were abundant in these agrarian communities because 1 stage of their life cycle is carried out in soil. Diseases such as typhoid fever and the plague were widespread then and have continued to afflict humans until recently.

Therefore, considering iron metabolism, 2 opposite forces may have operated throughout human evolution: first, the need to withhold iron from pathogens and, second, the need to acquire iron from food for the production of heme proteins and for other vital functions. These forces may have favored the emergence of opposing genetic traits. On the one hand, during times of iron sufficiency, an iron-withholding phenotype (from a genetic signature favoring hepcidin expression) would lead to iron sequestration, particularly in macrophages of the liver and spleen. This phenotype, has likely provided a selection advantage during microbial infections, and can be considered relatively "safe," because macrophages can retain large amounts of iron without harm. On the other hand, in times of low iron bioavailability, there was pressure for an iron-storing phenotype, with low hepcidin levels, leading to enhanced iron absorption, high serum iron, and more iron storage (accumulation) in parenchymal cells, mainly hepatocytes. This phenotype (from a genetic signature repressing hepcidin expression) may have provided a selection advantage during long periods of famine. However, in a context of iron sufficiency or repletion (and full erythroid activity), it becomes "unsafe" in that the enhanced iron absorption progressively saturates the buffering capacity of transferrin. Non–transferrin-bound and pro-oxidant forms of iron appear that ultimately accumulate in parenchymal cells and cause oxidative stress-mediated toxicity.

Hepcidin in Human Diseases Other Than Anemia of Chronic Disease and Hemochromatosis

Oxidative stress is, directly or indirectly, a pathogenic event in most, if not all, human diseases, and pro-oxidant iron plays a central role. This is particularly true for diseases of the liver, the main producer of hepcidin and a major storehouse of bodily iron reserves. This iron depot is mobilized, when needed; it accepts iron when serum levels are excessive. Excess iron deposition often accompanies metabolic, viral, and alcoholic liver diseases, and increasing data indicate that this phenomenon may contribute to liver disease progression, whereas iron deprivation may be beneficial. It is, therefore, not surprising that genetic factors that predispose to the iron-storing phenotype (such as the C282Y change in HFE, often in combination with other *HFE* polymorphisms), become pathogenic in the context of a chronic liver disease (Figure 2*A*). It is now also evident that nongenetic factors related to liver disease, such as alcohol, hepatitis C virus, or gluconeogenic/insulin resistance signals, directly modify hepcidin expression and cause hepatic iron trapping (in sinusoidal cells) or accumulation (in hepatocytes), depending on whether hepcidin is up-regulated or down-regulated, respectively. In these conditions, the systemic effects of hepcidin dysregulation (on transferrin saturation or erythropoiesis) may be marginal, but within the liver there may be substantial changes in iron status, oxygen radical production, and redox-sensitive intracellular signaling. This, in the end, may accelerate the course of the underlying liver disease.

Extrapolating the considerations reported to the general population, we can assume that the selection of genetic traits that predispose to an iron-withholding or iron-storing phenotype also impact on the course of other human diseases (Figure 2*B*). Genetic polymorphisms explains about one-half of the variation in serum iron markers,[13] and more and more loci associated with iron status (mainly related to genes involved in hepcidin regulation) are being identified by genomic research.[14]

In the context of the general population, an iron-withholding phenotype, an evolutionary remnant of our innate defense capabilities, is reactivated whenever nongenetic stress signals arise and trigger a defensive response by the liver, so that serum levels of the acute-phase protein hepcidin increase and iron is withheld, sometimes resulting in anemia. This is recapitulated by the induction of hepcidin during endoplasmic reticulum stress,[15] which can be found during chronic (micro)inflammatory or degenerative diseases, viral infections, and metabolic disorders (including obesity and diabetes). Even more interesting is the genetic makeup that predisposes individuals to a slight excess of (potentially pro-oxidant) serum iron (ie, the iron-storing phenotype), particularly when considering the risk for developing metabolic and cardiovascular

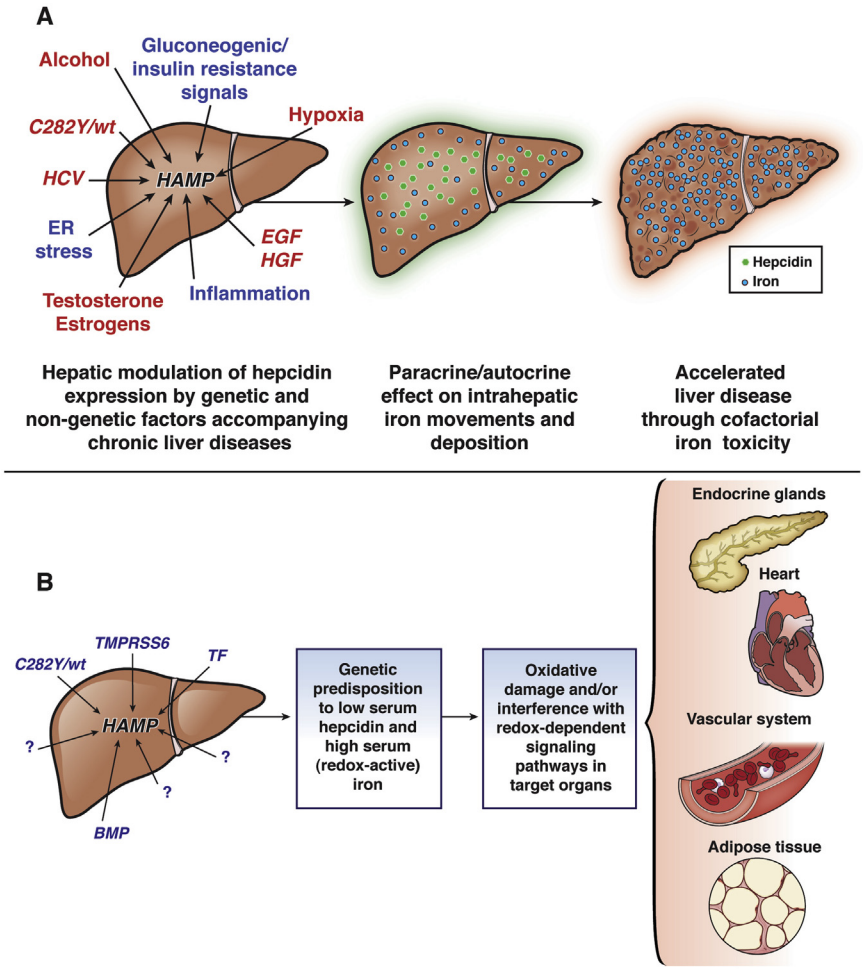

Figure 2. Effects of genetic and nongenetic hepcidin modifiers on the course of hepatic disorders and other human diseases. (*A*) Patients with chronic liver disease. Up- (*blue*) or down- (*red*) regulation of of hepcidin expression by genetic or nongenetic factors related to the underlying liver disease has paracrine or autocrine effects on iron traffic and deposition, exacerbating the disease. (*B*) General population. In persons with an iron-storing genotype, the tendency for increased serum iron favors oxidative stress within the circulation and in target organs and increases the risk for or exacerbates metabolic and cardiovascular disorders. *HAMP*, hepcidin antimicrobial protein gene; C282Y/wt, heterozygosity for the mutation in *HFE* that causes hemochromatosis; HCV, hepatitis C virus; EGF/HGF; epidermal and hepatocyte growth factors; ER, endoplasmic reticulum; TMPRSS6: Transmembrane protease, serine 6 (hepcidin inhibitor) gene; TF, transferrin gene; BMP, bone morphogenetic protein genes.

diseases (Figure 2*B*). In fact, in the general population, body iron stores are positively associated with the risk for type 2 diabetes.[16]

An Evolutionary Perspective: The "Iron-Thrifty" Genetic Model

Depending on the stability or instability of environmental conditions, evolution will or will not preserve a specific genetic trait in a population. In the presence of permanent environmental change, evolutionary discordance arises, and the average population genome moves to a new set point (ie, directional selection). However, in individuals retaining the original genome, evolutionary discordance will translate phenotypically into disease, increased risk of mortality, and reproductive failure.[17,18] Throughout evolution, whenever food excess has supplanted famine, humans have suffered major diseases. In the early ages, human genetics was optimized to store energy and micronutrients and to avoid starvation, and this genetic makeup remains largely the same today, because there has not been enough time, on an evolutionary scale, for the genetic adaptation to our modern, sedentary lifestyles and (over) nutritional habits.[18] The discordance between our ancestral genetic background and current lifestyle is thought to be the cause of several "diseases of civilization," such as diabetes, obesity, and cardiovascular pathologies.

The diabetes epidemic in certain populations today has been explained, in genetic terms, by J.V. Neel, who proposed the thrifty gene hypothesis.[19] According to Neel, our hunter–gatherer ancestors were able to survive long periods of famine thanks to a "thrifty genotype" that favored the storage of glucose and energy. However, in the modern setting of caloric excess, this thrifty genotype is thought to be responsible for the increased insulin levels and excessive energy stores in some patients with type 2 diabetes, resulting in insulin resistance and obesity.

There are numerous similarities between iron and glucose, insulin and hepcidin, and diabetes and hemochromatosis.[20] Translating Neel's model to iron, we can postulate that an iron-storing phenotype (an "iron-thrifty" genotype) may have reduced hepcidin secretion and favored iron storage to support hemoglobin synthesis and energy production and to avoid iron deficiency. A genetic disposition for increasing body iron stores (ie, a "thrifty" genetic makeup to save iron) may have been evolutionarily advantageous: persons who could form reserves during occasional times of plenty were able to survive long

periods of famine. In contrast, those with "iron-thrifty" alleles had no problem surviving in iron-poor environments, but they had a higher risk of iron overload in a context of iron repletion. Individuals carrying polymorphisms in hepcidin regulatory proteins, such as the C282Y mutation in HFE protein, are at risk of developing an iron-overload disease (eg, hemochromatosis) once exposed to lifestyle and dietary habits (eg, alcohol) that favor iron accumulation.[11]

In healthy individuals, blood iron levels are kept within a narrow range to avoid excess or deficiency. There is no doubt that iron deficiency remains a major health problem affecting 1 billion persons worldwide. Yet based on the considerations discussed, excess iron should also be regarded as a serious health problem, as is excess cholesterol. Unfortunately, we do not have effective therapies to decrease serum iron when in excess or to buffer the levels of pro-oxidant forms of iron in serum and cells. Phlebotomy is safe and effective in treating hemochromatosis, but it is impractical in cases of marginal iron excess associated with the human diseases discussed herein. Now that hepcidin has been identified as the key regulator of iron homeostasis, functioning as a central pathogenic factor in human diseases, novel medical applications based on this knowledge are emerging. It is hoped that, in the future, hepcidin-lowering or -enhancing agents will be developed to cure human diseases associated with dysregulated iron homeostasis.

ANTONELLO PIETRANGELO
University Hospital of Modena
Modena, Italy

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Conflicts of interest
The authors disclose no conflicts.

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