Noninvasive measurement of iron: report of an NIDDK workshop

Gary M. Brittenham and David G. Badman

An international workshop on the noninvasive measurement of iron was conducted by the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) on April 17, 2001, to assess the current state of the science and to identify areas needing further investigation. The workshop concluded that a clear clinical need is evident for quantitative, noninvasive, safe, accurate, and readily available means of measuring body storage iron to improve the diagnosis and management of patients with iron overload from such disorders as hereditary hemochromatosis, thalassemia major, sickle cell disease, aplastic anemia, and myelodysplasia, among others. Magnetic resonance imaging (MRI) potentially provides the best available technique for examining the 3-dimensional distribution of excess iron in the body, but further research is needed to develop means of making measurements quantitative. Biomagnetic susceptibility provides the only noninvasive method to measure tissue iron stores that has been calibrated, validated, and used in clinical studies, but the complexity, cost, and technical demands of the liquid-helium–cooled superconducting instruments required at present have restricted clinical access to the method. The workshop identified basic and clinical research opportunities for deepening our understanding of the physical properties of iron and iron toxicity, for further investigation of MRI as a method for quantitative determinations of tissue iron, especially in liver, heart and brain, and for development of improved methods and more widely available instrumentation for biomagnetic susceptibility. (Blood. 2003;101:15-19)

© 2003 by The American Society of Hematology

Introduction

Body iron burden is a principal determinant of clinical outcome in all forms of systemic iron overload, whether from transfusion (for thalassemia major, sickle cell disease, or aplastic, myelodysplastic, or other refractory anemias), from increased dietary iron absorption (hereditary hemochromatosis and other forms of primary iron overload), or both (refractory anemia with increased ineffective erythropoiesis). Accurate assessment of the body iron is essential for managing iron-chelating therapy in patients who are chronically transfused to prevent iron toxicity while avoiding the adverse effects of excess chelator administration. In hereditary hemochromatosis, determination of the magnitude of body iron stores permits identification of patients who would benefit from phlebotomy therapy from among those at genetic risk for the disease.

On April 17, 2001, an international workshop on the noninvasive measurement of iron was conducted by the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) to assess the current state of the science and to identify areas needing further investigation. Invited participants included representatives from centers worldwide that are active in the development of noninvasive measures of iron, clinicians who care for patients with iron disorders, and experts in the physics and chemistry of iron and in iron metabolism. The proceedings of the workshop are summarized below; more detailed information is available at the NIDDK website (http://www.niddk.nih.gov/fund/other/iron/index.html).

Clinical needs for measurement of iron

Iron is an essential nutrient required by every human cell. Under physiologic conditions, the concentration of iron in the human body is carefully regulated and normally maintained at approximately 40 mg Fe/kg body weight in women and approximately 50 mg Fe/kg in men, distributed between functional, transport, and storage compartments. Iron deficiency designates conditions in which body iron is decreased and arises from a sustained increase in iron requirements (usually from blood loss, pregnancy, or growth) over iron supply.1 Most cases of iron deficiency can now be detected by measurements of serum ferritin, serum transferrin receptor concentration, or both, determined in a sample of peripheral blood.2 Iron overload arises from a sustained increase in iron supply over iron requirements and develops with conditions in which the regulation of intestinal iron absorption is altered (hereditary hemochromatosis, refractory anemia with ineffective erythropoiesis), bypassed (transfusional iron overload), or both.1 Iron overload results primarily in an increase in storage iron held in ferritin and hemosiderin;2 functional iron is little affected. Whether derived from increased absorption of dietary iron or from transfused red blood cells, progressive iron accumulation eventually overwhelms the body’s capacity for safe sequestration of the excess. Symptomatic patients may have any of the characteristic manifestations of systemic iron overload: liver disease with the eventual development of cirrhosis and, often, hepatocellular...
carcinoma, diabetes mellitus, gonadal insufficiency and other endocrine disorders, arthropathy and increased skin pigmentation; iron-induced cardiomyopathy may be lethal. The prognosis in patients with iron overload is influenced by many factors, including the age at which iron loading begins, the rate and route of iron loading, the distribution of iron deposition between macrophage and parenchymal sites, the amount and duration of exposure to circulating nontransferrin-bound iron; ascabrate status; and coexisting disorders, especially alcoholism and viral hepatitis. Although ferritin and hemosiderin iron are almost surely not the species directly responsible for the adverse effects of iron, the overall magnitude of storage iron accumulation seems to be a principal determinant of clinical outcome in all forms of systemic iron overload.

The reference method for evaluating the extent of body iron excess in systemic iron overload is measurement of the hepatic storage iron concentration, recognizing that the exact relationship between hepatic iron and total body iron burden depends on the underlying disorder. The liver is the only storage compartment whose iron content is consistently increased with increased body iron stores; excess storage iron is detectable in reticuloendothelial (Kupffer cells) and parenchymal (hepatocytes) sites. Total body iron stores can be measured by quantitative phlebotomy, but this approach cannot be used in transfusion-dependent patients with iron overload and is generally acceptable only if the procedure provides therapeutic benefit. The measurement of plasma ferritin provides an indirect estimate of body iron stores, but the usefulness of this measure is limited by the many common clinical conditions in which plasma ferritin is not a reliable indicator of body iron. Inflammation, infection, liver disease, hemolysis, ineffective erythropoiesis, and ascorbate deficiency—common complications of hereditary hemochromatosis, transfusional iron overload, or both—all perturb serum ferritin levels independently of changes in total body iron.

Although liver biopsy with chemical analysis of tissue iron content provides the most quantitative direct measure of iron status generally available, the discomfort and risk of the procedure limit its acceptability to patients and preclude its frequent use in serial observations. The workshop concluded that physicians have a pressing need for quantitative means of measuring body storage iron that are noninvasive, safe, accurate, and readily available to improve the diagnosis and management of patients with iron overload, including those with hereditary hemochromatosis, thalassemia major, sickle cell disease, aplastic anemia, myelodysplasia, and other disorders. Priorities in clinical research in iron overload were identified as (1) developing, calibrating, and validating new methods or modifications of current methods for quantitative measurement of tissue storage iron that are noninvasive, safe, accurate, and readily available for clinical use; (2) examining the relationship between total iron burden and manifestations of iron toxicity in different forms of iron overload—for example, hereditary hemochromatosis and transfusional iron overload in thalassemia major, sickle cell disease, aplastic and myelodysplastic anemia; (3) assessing the value of noninvasive measurements of hepatic iron in guiding iron-chelating therapy in transfusional iron overload in patients with thalassemia major, sickle cell disease, aplastic anemia, and myelodysplastic anemia and in identifying patients who would benefit from phlebotomy therapy among those found to be at genetic risk for hereditary hemochromatosis; and (4) determining the relationships between the iron concentration in specific tissues and damage to the heart, liver, pancreas, other endocrine organs, and joints in different forms of iron overload—for example, in hereditary hemochromatosis and in transfusional iron overload with thalassemia major, sickle cell disease, and aplastic and myelodysplastic anemias.

Physical properties of iron

Iron in biologic systems is found with a variety of oxidation states, reduction potentials, magnetic properties, degrees of aggregation, solubilities, mobilities, and kinetic and thermodynamic properties toward free-radical generation. Each of these variables influences not only biologic activities and toxicities but also the detection and quantification of iron. Reduction-oxidation reactions are involved in the cellular uptake, transmembrane transport, and incorporation of iron into essential heme and nonheme enzymes for use and into ferritin for storage. Iron is likely to be found in the ferric oxidation state when tightly locked in place for purposes of transport or storage and in the ferrous oxidation state when a change of chemical environment is necessary. Iron can also be a detrimental catalyst in biologic free-radical oxidations, although the exact nature of the reactions involved remain uncertain. Iron-catalyzed free-radical production may take place through the Fenton and Haber-Weiss reactions, producing hydroxyl radical, or, perhaps, by reactions of ferrous iron with dioxygen, producing ferryl, perferryl radicals, or other reactive oxygen species. The recent extraordinary advances in our understanding of the cellular and molecular bases of iron uptake, transport, and storage have revealed new intricacies in the complexities of iron metabolism. Nonetheless, the specific toxic forms of iron have not been identified with certainty, and methods of measurement of the harmful species are lacking. Priorities in basic research in the physical properties of iron were identified as (1) characterizing the chemical nature of cytoplasmic iron in transit among different sites and routes of uptake, storage, use, and release by cells, including the redox reactions of iron storage and intracellular transport, the structure of iron in ferritin and hemosiderin, and the mechanisms of iron mobilization from these storage sites; (2) determining the forms and behavior of iron exported by cells for binding to circulating transferrin or appearing as nontransferrin-bound plasma iron; and (3) understanding mitochondrial iron metabolism and homeostasis and their disorders. Progress in these areas could lead to the identification of the precise species of iron that are responsible for toxicity and eventually to the means for their measurement and management.

Detection of iron overload by magnetic resonance imagining

MRI uses the magnetic properties of the body to provide detailed 3-dimensional images of any structure or tissue. Magnetic resonance measures the contribution of hydrogen nuclei (protons) to the net magnetization of the body within defined spatial regions. Hydrogen nuclei are a principal constituent of body tissues, mostly in water molecules and lipids. A spinning, charged hydrogen nucleus produces a dipole moment (magnetic field) that can interact with an external magnetic field. MRI instruments generate a strong and homogeneous magnetic field by using a large magnet made by passing an electric field through superconducting coils of wire. Patients are placed in a horizontal cylinder and are exposed to the magnetic field. At equilibrium, hydrogen nuclei in the body, which normally have randomly oriented spins, align in a direction
parallel to the magnetic field. The MRI machine then applies short electromagnetic pulses through a coil at a specific radio frequency (RF). The hydrogen nuclei absorb the RF energy and precess away from equilibrium (ie, alter the orientation of their spins). When the RF pulse is turned off, the precessing nuclei release the absorbed energy and return to equilibrium. An external RF coil detects the electromagnetic signals that are emitted as the nuclei return to equilibrium. The strength of the signals varies, depending on the applied RF magnetic fields. A sample returns to equilibrium in the longitudinal plane over a characteristic interval called the T1 relaxation time (the time constant for excited nuclei to dissipate excess energy to the environment). In the transverse plane, the return to equilibrium occurs over a characteristic interval called the T2 relaxation time (the time constant for excited nuclei to go out of phase with each other). Both T1 and T2 depend on the local environment of the hydrogen nuclei. These values may also be expressed as relaxation rates, R1 ( = 1/T1) or R2 ( = 1/T2). Rapid progress is being made in the development of improved MRI instrumentation, especially in the development of machines with higher magnetic field strength.13

With MRI, tissue iron is detected indirectly by the effects on relaxation times of ferritin and hemosiderin iron interacting with nearby hydrogen nuclei. Paramagnetic ferritin and hemosiderin iron shorten proton relaxation times, particularly T2, an effect termed susceptibility-induced relaxation,14 but a detailed, theoretical understanding of these effects is lacking.15 The interactions are complex and involve factors such as tissue hydration, water diffusion coefficient within the tissue, distribution of iron and water within the tissue examined, number of iron atoms per molecule of ferritin and hemosiderin (called the loading factor), and, because ferritin iron and hemosiderin iron have different effects on T1 and T2,16 relative proportion of these 2 iron storage materials. Loading factors within a tissue are not uniform; they differ with different types and amounts of iron excess.17 Studies of ferritin in solution in vitro show a clear effect of loading factor on relaxation times18; in tissue, clustering of ferritin within cells further complicates analysis.19 Moreover, conventional MRI measurements are also affected by the instrument used, the applied field strength, the repetition time used in the imaging sequence, the method used to analyze the relaxation curves, and other technical aspects of the measurement procedure. Comparison of absolute signal intensities from one MRI unit to another is unreliable because of substantial intermachine variation.20 In the absence of a theoretical understanding of the effects of iron on MRI, empirical efforts to estimate hepatic iron concentrations have used a variety of instruments, magnetic field strengths, imaging sequences (spin-echo, gradient recalled-echo), and parameters (T1 and T2 relaxation times, and signal intensity ratios as measured in proton, T1-, T2-, or T2*-weighted images), but no standard or generally accepted method has been adopted for clinical application. To date, MRI has been more useful as a screening technique for the detection of marked iron overload20 than as a means for quantitative measurement. In particular, with increasing iron concentrations, the signal intensity of the liver is reduced to such an extent that discrimination between different concentrations becomes impossible,21 at least with current technology.

New methods for using MRI to estimate tissue iron are under development, based on the inverse relationship between the susceptibility-induced relaxation, measured as R2, and iron concentration. One approach, applied in studies of brain iron, involves measuring the tissue relaxation rate (R2) in subjects in high- and low-field MRI instruments and then calculating the field-dependent R2 increase (FDRI), the difference between the R2 measured with the 2 MRI instruments. In tissue, only ferritin iron is known to increase R2 in a field-dependent manner, suggesting that the FDRI measure may provide a specific measure of this tissue iron pool.22,23 Another approach also involves the measurement of R2, but it uses a spin-echo imaging methodology to produce a series of images at different echo times. Using these images, a map of the entire liver is generated in which contrast is predominantly dependent on the R2 of the liver at different echo times.24 The advantage of this approach is that the results are independent of the MRI instrument used. Efforts to validate these approaches against chemical measurements of tissue iron are in progress.

At present, MRI provides a means of probing the 3-dimensional distribution of excess iron in the body, but further efforts are needed to make measurements quantitative. Priorities in basic and clinical research in the application of MRI to the measurement of tissue iron were identified as (1) acquiring an improved understanding of the contribution of ferritin and hemosiderin iron to magnetic resonance effects to guide the development of optimal methods for measuring relaxation times and susceptibility, including the best techniques for data acquisition, choice of field strength, selection of timing parameters, reduction of noise, identification of region of interest, and selection of analytic methods; (2) devising phantoms and other means for calibrating and validating iron concentration detected by MRI that could enhance standardization between different laboratories; and (3) developing new methods for noninvasive measurements of iron deposition in the heart, in endocrine tissue, and in specific areas of the brain to determine the role of abnormalities of brain iron in the pathogenesis of neurodegenerative disorders, including Alzheimer disease, amyotrophic lateral sclerosis, prion diseases, mitochondrial disorders, and Parkinson disease.

Detection of iron overload by magnetic susceptibility

The magnetic dipole moment is the central physical concept underlying MRI and magnetic susceptibility. Elementary particle magnetic moments are determined by quantum mechanical considerations and do not vary with time, magnetic field, or any other variable. In an applied magnetic field, all magnetic dipole moments experience torque, tending to align their axes. The disruptive effect of thermal fluctuations prevents all but a minute fraction of the available elementary particle moments in the human body from aligning in this way. Nonetheless, this minute field-induced fraction provides the diagnostic signals used by MRI and magnetic susceptibility. The magnetic susceptibility of a tissue is determined by the strength of the magnetic response evoked in the tissue by an applied magnetic field.6,25 This property is much simpler than the resonance behavior that results from the application of the oscillating magnetic fields used in MRI. While in a steady applied magnetic field, all materials respond with an induced magnetic field of their own. This response may be exploited diagnostically because the magnitude of the induced magnetic field varies greatly in different materials. In most human biologic materials, this induced field is diametrically opposed to the applied field. This diamagnetic response is so weak (approximately 10^-6 of the applied field) that its detection requires sensitive instrumentation, described below. By contrast, ferromagnetic materials (such as the common bar magnet) respond with an induced field as strong as or even stronger than the applied field and in the same direction. No
known human tissues are ferromagnetic. Intermediate between the diamagnetic and ferromagnetic extremes is the paramagnetic response of the iron in ferritin and hemosiderin that is in the same direction as the applied field with a strength approximately $10^{-4}$ of the applied field. This paramagnetic response is directly proportional to the number of iron atoms present in iron storage compounds. In a measurement of hepatic magnetic susceptibility in vivo, the opposing diamagnetic (tissue) and augmenting paramagnetic (ferritin and hemosiderin iron) responses are superimposed. By taking into account the small and nearly constant diamagnetic effect of the liver tissue, the observed resultant magnetic susceptibility may be used, in effect, to count the number of storage iron atoms present. The contributions of other paramagnetic materials (oxygen, deoxyhemoglobin, some trace metals) to the hepatic magnetic susceptibility are so small that magnetic measurements are highly specific for ferritin and hemosiderin iron. Thus, determinations of the magnetic susceptibility of the liver provide a direct measure of hepatic iron.

To date, measurements of hepatic magnetic susceptibility have required the use of superconducting magnetometers to measure the small change in magnetic field produced by the presence of storage iron in the liver (approximately 1 part in 1 billion of the applied field). Superconducting materials lose all electrical resistance below a certain temperature, called the transition temperature, or $T_C$. Until 1986, all known superconducting materials had transition temperatures near absolute zero and required a bath of liquid helium ($4.2^\circ$K) to keep their temperature below $T_C$. The recently developed “high-$T_C$” materials have $T_C$ values well above that of liquid nitrogen ($77^\circ$K). The superconducting quantum interference device (SQUID) is a superconducting loop incorporating a so-called weak link, known as a Josephson junction, that can function as the most sensitive and stable magnetic detector known. Since the development of the first SQUID susceptometer with the support of the NIDDK, the design of this instrument has been the basis for all commercially available instruments. In effect, the susceptometer provides an automated magnetic “biopsy” of liver ferritin and hemosiderin iron. In patients with iron overload, the results of susceptometric measurements of hepatic nonheme iron are quantitatively equivalent to those obtained by chemical analysis of tissue obtained by biopsy. Despite providing noninvasive, quantitative measurements of hepatic iron stores, clinical adoption of susceptometry has been limited, in part because of the cost and complexity of the instruments. At present, the only susceptometers in clinical use are at Columbia University, the University of Hamburg, Germany, and the University of Turin, Italy; a fourth instrument is being installed at the Children’s Hospital Oakland Research Institute, California. These instruments have now been used in studies of thousands of patients with iron overload.

At present, biomagnetic susceptometry provides the only noninvasive method for measurement of tissue iron stores that has been calibrated, validated, and used in clinical studies, but the complexity, cost and technical demands of the liquid-helium-cooled superconducting instruments now required have restricted clinical access to the method. The workshop identified basic and clinical research opportunities for deepening our understanding of iron toxicity, for further development of MRI as a method for quantitative determinations of tissue iron, especially in liver and brain, and for new approaches to methods for biomagnetic susceptometry.

### Summary and recommendations

Physicians have a pressing clinical need for quantitative means of measuring body storage iron that are noninvasive, safe, accurate, and readily available to improve the diagnosis and management of patients with iron overload, including those with hereditary hemochromatosis, thalassemia major, sickle cell disease, aplastic anemia, myelodysplasia, and other disorders. MRI provides a means of probing the 3-dimensional distribution of excess iron in the body, but further research is needed to develop means of making measurements quantitative. At present, biomagnetic susceptometry provides the only noninvasive method for measurement of tissue iron stores that has been calibrated, validated, and used in clinical studies, but the complexity, cost, and technical demands of the liquid-helium-cooled superconducting instruments now required have restricted clinical access to the method. The workshop identified basic and clinical research opportunities for deepening our understanding of iron toxicity, for further development of MRI as a method for quantitative determinations of tissue iron, especially in liver and brain, and for new approaches to methods for biomagnetic susceptometry.

**Note:** Since the workshop was held, additional publications on theoretical and practical efforts to develop noninvasive methods for measurement of tissue iron have appeared and are cited here for ready reference.

### Appendix: Invited workshop participants

**Introductory Remarks:** David Badman, DKUHD, NIDDK, National Institutes of Health, Bethesda, MD; Allen Spiegel, Director, NIDDK, National Institutes of Health, Bethesda, MD; Frank Somma, National President, Cooley’s Anemia Foundation, Inc.

**Session I. Clinical Needs for Measurement of Iron:** Gary Brittenham, Columbia University, New York, NY (Chair); Alan R. Cohen, Children’s Hospital of Philadelphia, PA; Roland Fischer, Universitätsklinikum Eppendorf, Hamburg, Germany; Harriet C. Isom, Pennsylvania State...
College of Medicine, Hershey; Elliott P. Vichinsky, Children’s Hospital of Oakland Research Institute, CA.

Session II. Physical Properties of Iron: Philip Aisen, Albert Einstein College of Medicine, Bronx, NY (Chair); Garry Buettner, University of Iowa, Iowa City; Dennis N. Chasten, University of New South Wales, Sydney, Australia; Alvin L. Crumbliss, Duke University, Durham, NC; Roland Frankel, California Polytechnic State University, San Luis Obispo, CA.

Session III. Detection of Iron Overload by MRI: Thomas Mareci, University of Florida, Gainesville (Chair); George Bartozek, University of Arkansas, Little Rock; Herbert Bonkovsky, University of Massachusetts, Worcester; Jeff Bulte, National Institutes of Health, Bethesda, MD; Peter Van Gelderen, National Institutes of Health, Bethesda, MD; Pierre Gillis, Université de Mon-Hainaut, Mons, Belgium; Tim St. Pierre, University of Western Australia, Nedlands, Australia; Evan S. Siegelman, University of Pennsylvania; Zhiyue J. Wang, Children’s Hospital of Philadelphia, PA.

Session IV. Detection of Iron Overload by Magnetic Susceptometry: David E. Farrell, Case Western Reserve University, Cleveland, OH (Chair); Paul N. Arendt, Los Alamos National Laboratory, NM; William Avrin, Quantum Magnetics, Inc., San Diego, CA; Gary M. Brittenham, Columbia University, New York, NY; Roland Fischer, University of Massachusetts, Boston, MA; Eppendon, Hamburg, Germany; Ronald B. Goldfarb, National Institute of Standards and Technology, Boulder, CO; Joseph L. Kirschvink, California Institute of Technology, Pasadena; Sankaran Kumar, Quantum Magnetics, Inc., San Diego, CA; Douglas Paulson, Tristan Technologies, Inc., San Diego, CA.

Session V. Discussion and Recommendations: Robert Balaban, National Institutes of Health, Bethesda, MD (Chair).

References


