Sex differences and steroid modulation of cardiac iron in a mouse model of iron overload

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Iron cardiomyopathy is the leading cause of death in transfusional iron overload, and men have twice the mortality of women. Because the prevalence of cardiac iron overload increases rapidly during the second decade of life, we postulated that there are steroid-dependent sex differences in cardiac iron uptake. To test this hypothesis, we manipulated sex steroids in mice with constitutive iron absorption (homozygous hemojuvelin knockout); this model mimics the myocyte iron deposition observed in humans. At 4 weeks of age, female mice were ovariectomized (OVX) and male mice were castrated (OrchX). Female mice received an estrogen implant (OVX + E) or a cholesterol control (OVX), whereas male mice received an implant containing testosterone (OrchX + T), dihydrotestosterone (OrchX + DHT), estrogen (OrchX + E), or cholesterol (OrchX). All animals received a high-iron diet for 8 weeks. OrchX, OVX, and OVX + E mice all had similar cardiac iron loads. However, OrchX + E males had a significant increase in cardiac iron concentration compared with OrchX mice ($P < 0.01$), whereas the OrchX + T and OrchX + DHT groups only trended higher ($P < 0.06$ and $P < 0.15$, respectively). Hormone treatments did not impact liver iron concentration in either sex. When data were pooled across hormone therapies, liver iron concentration was 25% greater in males than females ($P < 0.01$). In summary, we found that estrogen increased cardiac iron loading in male mice, but not in females. Male mice loaded 25% more hepatic iron than female mice regardless of the hormone treatment. (Translational Research 2014;163:151–159)

Abbreviations: ANOVA = analysis of variance; DHT = dihydrotestosterone; E = estrogen; GAPDH = glyceraldehyde-3-phosphate dehydrogenase; Hamp1 = hepcidin antimicrobial peptide 1; HFE = hemochromatosis; HJV = hemojuvelin; KO = knockout; LPI = labile plasma iron; mRNA = messenger RNA; OrchX = castrated; OVX = ovariectomized; PCR = polymerase chain reaction; T = testosterone; Zip14 = ZRT/IRT-like protein 14

Although the availability of iron is crucial to the body, iron excess can be lethal. For this reason, plasma iron concentration is tightly regulated by a negative feedback loop involving the hepatic hormone hepcidin.[1,2] Several iron-sensor proteins, including hemochromatosis (HFE) and hemojuvelin (HJV),[3,4] regulate hepcidin release according to transferrin saturation.[5] Hepcidin restricts cellular export of iron into the blood by the binding to and degrading of the iron exporter, ferroportin, the only known iron exporter

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Conflicts of Interest: Dr John C. Wood consults for Shire, ApoPharma, and Novartis, and has received speaker's honoraria and travel support from all three companies. The authors have no other financial or personal relationship with organizations that could potentially be perceived as influencing the described research. All authors have read the journal's policy on disclosure of potential conflicts of interest.

Partial funding for this study was provided by the National Heart, Lung, and Blood Institute (grant no. 1RC HL099412-01) and by Novartis.

Submitted for publication April 27, 2013; revision submitted August 7, 2013; accepted for publication August 16, 2013.

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1931-5244/$ - see front matter

http://dx.doi.org/10.1016/j.trsl.2013.08.004

AT A GLANCE COMMENTARY

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Background

Iron cardiomyopathy remains the leading cause of death for patients with iron overload, with cardiac iron accumulation peaking during adolescence. Mortality rate is twice as great for men than for women, suggesting a role for sex steroids in cardiac iron accumulation and toxicity.

Translational Significance

Estrogen increased cardiac iron in males but not females, indicating organizational differences in steroid sensitivity. Increased cardiac iron loading in peripubertal and postpubertal males may contribute to their poorer survival.

in the body.[6] When ferroportin has been degraded, iron can no longer enter the bloodstream from its main sources, the enterocytes of the duodenum and macrophages that recycle old red blood cells. Hepcidin controls the flux of iron such that all labile forms can be bound safely by the carrier protein transferrin,[7] preventing unregulated redox chemistry in the circulation.

Primary iron overload occurs when there is a deleterious mutation in the iron regulatory system (e.g., HFE or HJV).[8,9] This disease is characterized by a blunted hepcidin response, resulting in high plasma iron and high transferrin saturation.[10,11] Iron overload can also occur in response to chronic blood transfusions, a condition known as secondary iron overload.[12] Some patients with hemoglobinopathies (e.g., β-thalassemia) require chronic blood transfusions every 3–4 weeks, with each transfusion containing 400–600 times the daily absorption of iron.[13] Because humans have no way of upregulating iron excretion, transfusional iron eventually overwhelms the hepcidin regulatory system, saturates transferrin binding capacity, and deposits in the endocrine glands and the heart. Although the exact transporters responsible for extrahepatic iron overload are not known, L-type calcium channels, T-type calcium channels, and ZRT/IRT-like protein 14 (Zip14) zinc channels have been implicated in previous animal studies.[14-16]

Thalassemia is the most common genetic disease worldwide, with a very high prevalence in Asia.[17] Although chronic blood transfusions correct patients' anemia, they produce severe iron overload that can become lethal during the second decade of life.[18] The leading cause of death in these patients is iron-mediated cardiomyopathy.[19] Women with thalassemia have a 2:1 survival advantage compared with men with the disease[20]; similar disparities in disease severity have been documented in hereditary hemochromatosis.[21] In thalassemia, the onset of cardiac iron overload is greatest during puberty.[18] Thus, we postulated that sex steroids might modulate cardiac iron loading, with androgens and estrogens acting antagonistically.

Response to sex steroids can be classified as organizational or activational.[22,23] Organizational effects persist even if steroids are later removed. An example would be steroidal actions *in utero* that determine male or female genitalia. Activational effects can occur throughout the life span and are more reversible, such as increased muscle mass caused by testosterone and the bone-strengthening effects of estrogen. We sought to determine whether sex steroids have organizational effects and/or activational effects with respect to cardiac iron loading. Experiments were performed in gonadectomized mice, allowing for hormonal regulation by subcutaneous implants. Because testosterone can act through both androgenic and estrogenic mechanisms, we studied cardiac iron responses to testosterone, dihydrotestosterone (DHT), and estrogen.

To evaluate possible sex differences in cardiac iron overload, we used the HJV knockout (KO) mouse, a model of juvenile hemochromatosis, supplemented with dietary iron. Our goal was to mimic the cardiac iron exposure found in transfusional siderosis. Although HFE mutations are more common than HJV mutations in humans, cardiac iron accumulation does not occur on an experimentally feasible timescale (many decades). The HJV mice continuously absorb iron from their diet, overcoming the high spontaneous iron elimination mechanisms found in rodents, and develop myocyte iron levels similar to that found in human adolescents with transfusional siderosis or juvenile hemochromatosis.[24] Although high total cardiac iron levels can also be produced by iron dextran injections in rodents, iron is retained preferentially in cardiac phagocytic cells (which humans lack) rather than myocytes.[25] Without a means to track changes in myocyte and phagocytic cell iron burdens separately, response of iron dextran models to therapies can be difficult to translate to humans. Thus, we believe that severe hemochromatosis mutants, such as HJV KO, represent the closest practical mimic to cardiac siderosis experienced in transfusional iron overload.

METHODS

Animals. Mice were housed in the Animal Care Facility of Children's Hospital Los Angeles. All studies were carried out with approval of the Institutional Animal Care and Use Committee of Children's Hospital Los Angeles. HJV KO mice were used to induce dietary iron overload; these mice have the background strain of