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Iron and a Man's Reproductive Health: The Good, the Bad and the Ugly

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Abstract

Purpose of Review: To discuss the physiologic and pathologic effects of iron on men's reproductive health.

Recent Findings: Iron overload diseases are associated with hypogonadotropic hypogonadism, infertility and sexual dysfunction in men. Recent findings have elucidated the roles by which iron may affect the male reproductive axis.

Summary: Iron is requisite for life. Iron can also catalyze the production of reactive oxygen species. To maintain balance, the human body tightly regulates dietary iron absorption. Severe iron overload disorders—e.g., hereditary hemochromatosis and β-thalassemia—occur when these regulatory mechanisms are deficient. While iron is necessary, the male reproductive system is particularly sensitive to iron overload. Hypogonadotropic hypogonadism, infertility and sexual dysfunction commonly occur if excess iron from iron overload disorders is not removed. The average male in the United States consumes significantly more iron than needed to replace daily losses. How this degree of iron loading may affect one's reproductive health remains less clear; however, there is evidence that even this level may have adverse effects.

Keywords

iron overload; hypogonadotropic hypogonadism; male infertility; erectile dysfunction; oxidative stress; anejaculation

Introduction

In the body, iron presents a paradox: its oxidative-reductive potential is an essential component of many enzymes and required for life, yet that same potential can catalyze the production of reactive oxygen species and lead to cellular death. This dichotomy is

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particularly true in the male reproductive system, where there is a precipitous balance between sufficient and too much iron. This review provides a brief overview of body and cellular iron regulation prior to delving into the role of iron—both physiologic and pathologic—in the regulation of reproductive hormones, fertility and sexual function in men.

Iron Regulation in the Human Body

In the United States, the average male consumes 10–15 times more iron than needed to replenish incidental losses [1]. The human body lacks a mechanism for eliminating excess iron [2]. To prevent overload, dietary iron absorption must be tightly regulated. Excess iron must also be sequestered to minimize the generation of reactive oxygen species. Highly conserved mechanisms exist to ensure adequate iron supplies while preventing excess concentrations. Severe iron overload occurs when these mechanisms are defective or bypassed, as in hereditary hemochromatosis and β-thalassemia, respectively [3, 4].

To accomplish this requisite tight regulation, mechanisms exist at both the cellular and whole organism level (reviewed in [2, 4]). The primary mechanism by which cells obtain iron is mediated by transferrin receptor 1 (TFRC). Under normal circumstances, the majority of serum iron is bound to transferrin. Transferrin binds TFRC on the cell surface and the receptor complex is endocytosed. Acidification of the endosome releases the iron from transferrin and it passes into the cell through divalent metal transporter 1 (DMT1). Iron that is not immediately used is sequestered in ferritin, a clamshell-like protein that protects the cell from iron's oxidative potential.

Cellular iron homeostasis is regulated by iron response elements (IREs) in the mRNAs of TFRC, DMT1 and ferritin. IREs post-transcriptionally regulate mRNA levels and translation of these genes [2]. When intracellular iron levels are low, TFRC and DMT1 mRNAs are stabilized, resulting in increased translation. Under high iron conditions, ferritin mRNA translation increases and TFRC and DMT1 mRNAs are degraded, thereby increasing iron storage and decreasing uptake. Thus, when there is cellular need for iron, the uptake mechanisms increase; when there is sufficiency/excess iron, these mechanisms decrease and storage capability increases. Through this regulation, cellular iron homeostasis is maintained.

While all cells express the machinery to take up iron, relatively few cells express the iron export protein, ferroportin [1]. Ferroportin-expressing cells are predominantly involved in the maintenance of whole body iron stores. For example, ferroportin expression in duodenal enterocytes allows dietary iron to transverse the gut epithelium and enter the blood stream. In macrophages, it releases iron recycled from senescent red blood cells back into circulation. Hepatocytes are the primary storage site of excess body iron. Ferroportin allows release of the iron from hepatocytes when serum iron levels decline. Ferroportin is also regulated by IREs and its expression is inhibited under low intracellular iron conditions.

At the whole-body level, iron stores are coordinated by hepcidin [1]. Hepcidin is a circulating peptide produced by the liver in response to high iron levels. It binds ferroportin, blocking iron egress, and leads to its internalization and degradation [5, 6]. Thus, under high

body iron conditions, serum hepcidin levels rise and ferroportin expression decreases. This prevents absorption of dietary iron and impairs release of iron from hepatocytes and macrophages, thereby reducing serum iron levels. When serum iron levels fall, hepcidin expression decreases and ferroportin increases, resulting in release of iron from hepatocytes and macrophages and increased absorption from the diet.

Severe iron overload occurs when these regulatory mechanisms fail. In hereditary hemochromatosis, hepcidin is uncoupled from body iron stores [3]. Thus, hepcidin levels remain low and iron continues to be absorbed from the diet despite extreme iron overload. In β-thalassemia, chronic blood transfusions bypass the hepcidin-ferroportin regulation of dietary iron uptake, resulting in massive iron loading.

Iron-containing enzymes are involved in many cellular processes including DNA replication, intracellular signaling, biosynthesis and modification of molecules, detoxification of reactive oxygen species and energy production. As such, iron is important for spermatogenesis and steroid production. Sertoli cells express TFRC, DMT1, ferritin and ferroportin [7]. Interestingly, iron regulation within the Sertoli cell is particularly complex as there is a polarized gradient of iron concentrations that decreases toward the lumen of the seminiferous tubules. This likely reflects the decreasing need for iron and increasing need to protect developing spermatozoa from the oxidative effects of iron [7]. It remains unclear whether Sertoli cells also express ferroportin protein. As the blood-testis barrier is impermeable to iron, however, ferroportin expression would be expected in order to supply iron for spermatogenesis. Less is known about iron regulation in Leydig cells; however, the iron uptake and storage proteins would also be expected to be present.

Iron and Sex Hormones

Profoundly low testosterone levels are a common finding severe iron overload. In one study, hypogonadism was present in 65% of men with β-thalassemia, while hypothyroidism was only present in 4% [8]. Furthermore, male children with β-thalassemia will often fail to enter puberty unless the iron is chelated [9, 10].

The pituitary is particularly sensitive to iron. MRI studies demonstrate increasing iron deposition and decreasing pituitary volume as body iron stores increase [11, 12]. Pituitary iron levels are negatively correlated with serum testosterone levels [11]. Autopsy studies in iron overloaded men revealed that iron deposition is specific to the gonadotrophs [13, 14]. Therefore, hypogonadism in both hereditary hemochromatosis and β-thalassemia is primarily due to decreased luteinizing hormone (LH) production—i.e., hypogonadotropic hypogonadism.

There is evidence that iron may negatively regulate LH production prior to the destruction of the gonadotrophs. As mentioned previously, chelation of iron in male children with β-thalassemia can allow them to enter puberty if performed early and consistently [9, 10]. Likewise, hypogonadotropic hypogonadism can be reversed by phlebotomy in young men with hereditary hemochromatosis [15–17]. It is not reversible in young adults with poorly

treated β-thalassemia or older men with hereditary hemochromatosis, suggesting that the hypogonadism becomes permanent once the gonadotrophs are lost.

Pituitary iron deposition is not limited to severe iron overload diseases, though. Autopsy studies reveal that beginning in the third decade of life, gonadotrophs show progressively increasing iron deposition in the general population [18]. The gonadotrophs are replaced with fibrosis as pituitary iron levels increase. These data indicate that iron can have toxic effects impacting pituitary function in the general population.

It remains unclear whether the low testosterone seen in these men is strictly due to decreased LH production, or if there is a concomitant testicular testosterone production defect. MRI of the testes also demonstrates increased iron deposition and human chorionic gonadotropin (hCG)-stimulated testosterone production is abnormal in some of these males [19, 20]. For example, 6 months of hCG therapy failed to increase testosterone levels above 2 nmol/L (58 ng/dL) in 42% of adolescents with delayed puberty due to β-thalassemia, whereas all the adolescents with constitutional delayed puberty responded [20]. Of the children with β-thalassemia, serum ferritin levels were significantly higher in the non-responders compared to the responders, suggesting that more severe iron overload results in a compound testicular and pituitary defect. In rats, chronic iron injections result in testicular iron deposition, predominantly within the Leydig cells [21]. Whether this results in an isolated testosterone production defect remains unknown.

The Yin-Yang of Iron and Testosterone

While these data indicate that iron decreases LH and testosterone levels, testosterone conversely regulates body iron levels by inhibiting hepcidin synthesis [22]. Thus, increasing testosterone levels increase dietary iron absorption and body iron stores. This testosterone effect is robust and overrides the iron regulation of hepcidin.

The interplay of iron and testosterone it may be particularly relevant to the general population. First, the time of highest testosterone levels (puberty) correlates with the time of highest iron demand. Thus, testosterone's ability to override the regulatory mechanisms to maximize iron absorption would beneficially increase iron stores needed for rapid growth. Second, the effects of iron on the pituitary may help prevent overload. Specifically, if testosterone increases iron absorption and the body is unable to eliminate excess iron, then the ability of iron to downregulate LH would provide a negative feedback mechanism to help maintain iron homeostasis. Such a mechanism is thought-provoking as it suggests that age-related hypogonadism could be a *physiologic* response to increasing body iron stores—an attempt to decrease iron absorption by decreasing LH and testosterone levels.

Another interesting association is the overlap between the clinical presentation of hereditary hemochromatosis and the side effects of exogenous testosterone use. Shared features include hypogonadotropic hypogonadism, infertility, polycythemia, heart failure and liver dysfunction. From a physiologic perspective, this association makes sense: exogenous testosterone administration decreases hepcidin levels, uncoupling hepcidin from body iron stores. This uncoupling is the physiologic basis for hereditary hemochromatosis. In essence,

testosterone supplementation (particularly high dosage use/abuse), physiologically causes hemochromatosis. Thus, some of the side effects of supplemental testosterone use may be directly related to iron overload.

The above hypotheses are novel and have yet to be directly tested in humans. Nonetheless, dietary iron overload significantly decreases LH levels in wild-type mice, with a trend toward decreased testosterone levels [23]. Additionally, serum ferritin levels are negatively associated with serum testosterone levels in healthy adult men (J. S. Gabrielsen, manuscript in preparation), indicating that clinically significant reciprocal regulation may occur in the absence of hemochromatosis or thalassemia.

Iron and Fertility

Iron overload also negatively impacts follicle stimulating hormone (FSH) production and male fertility. Gonadotropin-releasing hormone (GnRH)-stimulation tests in men with β-thalassemia, however, indicate that FSH production may not be as sensitive to iron loading as LH [24]. The role of iron in male infertility is complicated, as many men with severe iron overload will also have low testosterone levels. It may be difficult to determine whether altered semen parameters are directly due to iron overload, reactive oxygen species, hypogonadism, or a combination of these factors. The balance between sufficient and too much iron may be more stringent in male fertility than in testosterone production, though, as iron is not only necessary for enzymatic function, but even low levels of oxidative stress are also necessary for sperm development and function [25–27].

The need for iron in male fertility is underscored by the observation that seminal plasma is the only known bodily fluid where iron is actively secreted. Transferrin is one of the most abundant proteins in seminal plasma, comprising up to 5% of the total protein [28]. Both FSH and testosterone induce transferrin mRNA in Sertoli cells, and approximately 80% of seminal plasma transferrin is produced by the testicle [28, 29]. Thus, seminal transferrin has been used as a marker of Sertoli cell function [28, 30].

Seminal plasma iron concentrations are reportedly between 1.0 and 3.7 μg/ml in healthy, normozoospermic men [31]. The literature regarding seminal plasma iron concentrations in men with abnormal semen analyses is inconsistent for the general population [31–33]. A small study of men with transfusion-dependent β-thalassemia, however, reported elevated seminal plasma iron concentrations in five of six men, with concentrations approximately 5–10 times higher than the reference range [12]. Of the five men, semen parameters were abnormal in four [12]. DNA damage is also higher in men with β-thalassemia compared to normozoospermic controls [34].

Naes et al investigated the effects of supplemental iron on spermatozoa from normozoospermic controls. Sperm motility, vitality and DNA integrity increased with a supplemented concentration of 2.0 μg/ml. Above 4.0 μg/ml, however, both motility and vitality were impaired [35]. There is close overlap between these supplemental iron concentrations the normal range of seminal plasma iron concentrations (1.0 to 3.7 μg/ml) [31].

Interestingly, spermatozoa were the first mammalian cell identified to produce hydrogen peroxide [36]. While usually reported to adversely affect fertility, reactive oxygen species play a necessary and important role in sperm development and function. In mice, physiologic levels of hydrogen peroxide promote, while antioxidants suppress spermatogonial stem cell self-renewal and proliferation [25]. Additionally, low level generation of reactive oxygen species through NADPH oxidase and hydrogen peroxide improve sperm DNA integrity and enhance sperm-oocyte fusion [26, 27].

Iron may also play an important role in the mitochondria, as mutations in mitoferrin (which transports iron across the inner mitochondrial membrane) results in sterility in male fruit flies, with 100% penetrance [37]. Humans have two orthologs to mitoferrin; however, the clinical relevance of these findings to humans remains unknown [38]. Impaired mitochondrial function would be expected to be harmful given the extensive mitochondrial biogenesis that occurs during spermatogenesis. Additionally, synthesis of the iron-sulfur (heme) clusters that form the catalytic core of iron-containing proteins occurs in the mitochondria and is affected by mutations in the mitoferrin genes [38].

Too much iron, may negatively impact fertility. For example, varicoceles are common and more prevalent in the infertile population. In rats, experimentally induced varicoceles increase testicular iron deposition. Sperm count and motility decrease while DNA damage and markers of oxidative stress increase [39]. It remains unknown whether varicocele induces iron deposition in human testes; however, men with grade III varicoceles have elevated levels of malondialdehyde (a marker of lipid peroxidation), consistet with elevated oxidative stress [40].

Severe iron overload is consistently associated with impaired spermatogenesis. In mice, repeat exposure to iron oxide nanoparticles decreases sperm counts and worsens morphology [41]. A study of 14 eugonadal, β-thalassemic men found 1 to be azoospermic, 3 oligozoospermic, 5 asthenozoospermic and 2 teratozoospermic. There were no abnormalities noted in the controls [19]. Sperm DNA fragmentation was higher among men with β-thalassemia and positively correlated with serum ferritin levels [19].

These findings may be explained by the oxidative-reductive potential of iron. Iron can catalyze the Fenton reaction, which converts hydrogen peroxide into the peroxyl radical, one of the most damaging of the free radicals [42]:

$$H_2O_2 + Fe^{2+} \rightarrow Fe^{3+} + OH^- + OH^\circ$$

The peroxyl radical is particularly reactive with unsaturated fatty acids—which are abundant in sperm—and result in lipid peroxidation [43]. Therefore, while low levels of hydrogen peroxide may be beneficial and intentionally generated by spermatozoa, the presence of iron overload may make any level harmful.

Additionally, there are varying reports in the literature regarding persistent infertility in men with hemochromatosis and β-thalassemia following iron depletion. One case report of a 36-year-old male with hereditary hemochromatosis undergoing phlebotomy had return of

spermatogenesis and spontaneous pregnancy despite ongoing testosterone therapy [16]. This appears to be more the exception than the norm, however, as a study of assisted reproductive technologies (ART) outcomes in men with β-thalassemia found that gonadotropin +/− GnRH therapy was only able to induce spermatogenesis in six of nineteen men, with no spontaneous and only two successful IVF-ICSI pregnancies attained [44]. A separate study of β-thalassemic men reported gonadotropin treatment improved testosterone levels and semen parameters, but only one subject was able to father a child [24].

Iron and Sexual Function

Sexual dysfunction (i.e., erectile dysfunction and ejaculatory dysfunction) is a prominent complaint of men with iron overload disorders. This association is also complex, however, as there is often comorbid hypogonadotropic hypogonadism, diabetes and decreased libido in these men. Nonetheless, there is evidence that iron overload may have a direct effect on sexual function.

A retrospective cohort study found that men with non-transfusion-dependent β-thalassemia (who have a milder iron overload and lower risk of hypogonadism than transfusion-dependent men) still have a 4.6-fold increased risk of developing erectile dysfunction compared to non-thalassemic controls [45]. A case series of eugonadal, β-thalassemic men with erectile dysfunction found an impaired response to intraurethral alprostadil, suggesting a hypogonadism-independent effect of iron [46]. Therapeutic phlebotomy can reverse erectile dysfunction in young adults diagnosed with hereditary hemochromatosis, suggesting the iron effect may be reversible if the excess iron is removed prior to permanent cell damage [15, 16].

Penile erection is dependent upon vascular smooth muscle relaxation in the corpora cavernosa, which is induced by nitric oxide release by the cavernosal nerve [47]. Soluble guanylyl cyclase (sGC) is the principle target of nitric oxide signaling and is a heme-containing protein [48]. While sGC lacking the heme core still has basal cyclase activity, it loses its responsiveness to nitric oxide [49]. Corporal arteries in mice expressing this heme-lacking sGC do not relax in response to cavernosal nerve stimulation or nitric oxide donors [49]. Thus, iron at the core of sGC is critical for normal erectile function.

The mesenteric arteries and aortas—and presumably the cavernosal arteries—from iron overloaded rats have increased contractile response to phenylephrine, decreased nitric oxide availability and increased superoxide production compared to saline-injected rats [50, 51]. Likewise, arteries from iron overloaded mice had decreased relaxation in response to acetylcholine, again suggesting a decrease in nitric oxide availability [52]. Increased contractility and impaired relaxation in the cavernosal arteries would be anticipated to culminate in erectile dysfunction.

The negative effects of iron appear to be mediated by increased reactive oxygen species. Reactive oxygen species such as superoxide deplete nitric oxide by reacting with it to form peroxynitrates [49]. Additionally, they can oxidize and inactivate the heme moiety of sGC, impairing its response to the remaining nitric oxide [49]. Inhibition of reactive oxygen

species-generating enzymes and treatment with free radical scavengers attenuate the iron effects [50–52].

Like hydroxyl radicals, peroxynitrates are very reactive with lipid membranes and can induce permanent damage in smooth muscle cells and nerves. This may be a significant cause of erectile dysfunction [53]. One study reported idiopathic polyneuropathy in 26% of non-diabetic men and women with hereditary hemochromatosis [54]. The etiology of this polyneuropathy remains unknown; however, it suggests that nerve damage may contribute to erectile dysfunction in iron overload disorders.

Proper nerve signaling is important for ejaculatory function. The effects of iron overload on ejaculatory function are also complex, given that diabetes is associated with iron overload, polyneuropathy and anejaculation. Nonetheless, one study reported anejaculation in 13% of men with hereditary hemochromatosis compared to 0% of controls with similar duration and severity of diabetes [55]. One of the subjects complaining of anejaculation did not have diabetes, again suggesting that iron and/or iron-induced neuropathy may have a causal role [55]. Similarly, a mail survey of men with hemochromatosis found that 39% of respondents reported anorgasmia and 20% reported anejaculation, although there was limited data regarding the prevalence of diabetes and hypogonadism in this population [56]. Nonetheless, it appears that severe iron overload may also negatively impact ejaculatory function.

Conclusion

Iron impacts all aspects of men’s reproductive health, although not necessarily in a negative manner. Iron and even reactive oxygen species are necessary for proper sperm development and function. Nonetheless, severe iron overload—as found in hereditary hemochromatosis and β-thalassemia—negatively affects serum reproductive hormones, male fertility and sexual function both directly and indirectly through comorbidities such as diabetes. The extent that milder perturbations in body iron levels affect male reproductive health in the general population remains largely unknown. Understanding these effects, however, is critical to men’s reproductive health and may provide new avenues for treating—and even preventing—age-related hypogonadism, infertility and sexual dysfunction in the general population.

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