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Acute iron overload leads to hypothalamic-pituitary-gonadal axis abnormalities in female rats

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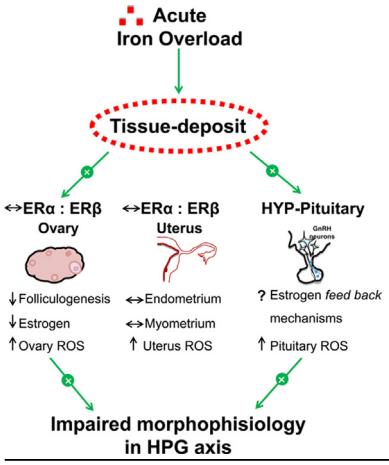
HIGHLIGHTS

- Acute iron overload accumulated in female rat hypothalamic-pituitary-gonadal axis
- Acute iron overload impairs hypothalamic-pituitary-gonadal axis morphophysiology
- Acute iron overload increased oxidative stress in pituitary, uterus and ovary *in vivo*.

GRAPHICAL ABSTRACT



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ABSTRACT

Iron plays a critical role in a mammal's physiological processes. However, iron tissue deposits have been shown to act as endocrine disrupters. Studies that evaluate the effect of acute iron overload on hypothalamic-pituitary-gonadal (HPG) axis health are particularly sparse. This study demonstrates that acute iron overload leads to HPG axis abnormalities, including iron accumulation and impairment in reproductive tract morphology. Female rats were treated with iron-dextran (Fe rats) to assess their HPG morphophysiology. The increasing serum iron levels due to iron-dextran treatment were positively correlated with higher iron accumulation in the HPG axis and uterus of Fe rats than in control rats. An increase in the production of superoxide anions was observed in the pituitary, uterus and ovary of Fe rats. Morphophysiological reproductive tract abnormalities, such as abnormal ovarian follicular development and the reduction of serum estrogen levels, were observed in Fe rats. In addition, a significant negative correlation was obtained between ovary superoxide anion and serum estrogen levels. Together, these data provide in vivo evidence that acute iron overload is toxic for the HPG axis, a finding that may be associated with the subsequent development of the risk of reproductive dysfunction.

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1. Introduction

Iron is an essential metal of life that plays important physiological roles in mammals, such as the production of red blood cells, the transport of gases, the production of energy, acid nucleic synthesis and the cell division process (Andrews, 2000; Ganz, 2013). In mammals, iron is primarily absorbed by the gastrointestinal tract (GIT) and it is associated with their diet and haemocatheresis sources to control body iron homeostasis (Iancu, 1982; Crawford, 1995; Donovan et al., 2005). In addition, daily iron loss usually occurs due to cell desquamation in skin and intestinal mucosa (Green et al., 1968). In females, menstrual or estrous cycles are physiological processes that can cause reduced in the iron levels from a blood source (Green et al., 1968; Latunde-Dada et al., 2006; Oliveira et al., 2014).

Several studies have shown that the signalling pathways responsible for iron elimination, which are not well understood, contribute to iron tissue-deposits and primary or secondary physiology dysfunction development (Latunde-Dada et al., 2006; Madiwale and Liebelt, 2006; Siddique and Kowdley, 2012; Brewer et al., 2014). Primary iron overload occurs when there is a genetic disorder in the iron regulatory system (Feder et al., 1996). For instance, the hereditary hemochromatosis was characterized by abnormal iron metabolism, leading an excessively iron absorption and store in the body (Bolondi et al., 2010). Iron overload can also occur in the ineffective erythropoiesis, sideroblastic anemia, primary liver disease, thalassemia, dyserythropoietic anemia, and Sickle cell disease, hematological diseases that needing chronic blood transfusions, a condition known as secondary iron overload (Weatherall and Clegg, 1996; Porter, 2009). Regarding multiple cellular disorders that are iron deposit-induced, various studies have demonstrated that an oxidative stress process plays a critical role in the organ damage development via iron overload (Lucesoli et al., 1999; Shazia et al., 2012). Therefore, iron accumulation may cause cellular toxicity via production of free oxygen species (ROS) production, which reacts with free iron ions from iron metabolism processes (Lucesoli and Fraga, 1995; Kramer et al., 2012).

Previous studies of iron overload models reported that iron can accumulate in different tissue/organs, such as blood, heart, liver, spleen, bone marrow, muscles and endocrine glands (Lucesoli and Fraga, 1995; Lucesoli et al., 1999; Guan et al., 2013; Musumeci et al., 2014). Consequently, chronic iron overload and tissue accumulation were able to contribute to the development of endocrine disorders, such as diabetes mellitus, hypothyroidism and gonadal dysfunction (Lucesoli and Fraga, 1995; Lucesoli et al., 1999; Singer et al., 2011). In mammalian hypogonadism, a reduction of pituitary gonadotropin hormones levels, impairment in sexual hormone metabolism, estrogen receptor (ER) function, gonadal morphophysiology and others dysfunctions may cause the harmful effect of chronic iron deposit on the hypothalamic-pituitary-gonadal (HPG) axis, which may explain the impairment of reproductive function in female β-thalassemia patients or other iron overload model (Luck et al., 1995; Singer et al., 2011; Roussou et al., 2013). However, the toxicology effect of iron overload in reproductive function is not fully understood. Several health risk assessments have largely been based on iron overload animal models, as result of chronic iron accumulation in their tissue (Safarinejad, 2008; Singer et al., 2011; Asano, 2012).

Despite these advances using the iron overload models (Lucesoli et al., 1999; Skordis et al., 2004; Al-Rimawi et al., 2005; Singer et al., 2011), few studies have investigated the effect of acute iron overload signalling at the HPG axis control on mammalian reproductive function. Therefore, this present study aimed to investigate if acute iron overload can interfere on the HPG axis of female rats. The implication of identifying the precise signalling of iron on the reproductive axis significantly contributes to our continuously evolving understanding of the HPG axis and identifies potential targets for iron overload in reproductive function.

2. Materials and methods

2.1. Experimental animals and acute iron treatment

Adult female Wistar rats (200–230 g, 12 weeks old) were kept in polypropylene cages at a controlled temperature and humidity and were exposed to 12 h (-h) of a light/dark cycle. Rats were obtained and maintained at the Central Animal Facility at the Health Sciences Center, Federal University of Espirito Santo (Vitoria, ES, Brazil) and were fed with a standard rat chow diet and received water *ad libitum*. The animals were weighed, randomly assigned to four experimental groups and were injected intraperitoneally (*ip*) with iron-dextran (Ferrodex®, Fabiani Ltda, São Paulo, SP, Brazil) at doses of 250 mg/kg (Fe250, $n = 10$), 500 mg/kg (Fe500, $n = 10$), or 1000 mg/kg (Fe1000, $n = 10$) or were injected with saline solution (Control, $n = 10$). The doses and route were selected according to a previous study described by Lucesoli et al. (1999). Briefly, the rats were treated *ip* with 1.5 mL of iron-dextran at doses of 250, 500 or 1000 mg/kg body weight (referred to as Fe rats); after 24-h, we assessed their HPG axis function. The control group received the vehicle (saline) following the same protocol used for the Fe rats. All assessments were performed 24-h after the single injection of iron or saline. The care and use of experimental animals conformed with the Brazilian Guidelines for the Care and Use of Animals for Scientific and Educational Purposes and the National Institutes of Health guidelines (Guide for the Care and Use of Laboratory Animals) and were approved by the Institutional Ethics Committee on Animal Use (010/2013CEUA-UFES).

2.2. Iron levels assessment

Serum iron concentrations were performed, blood samples were collected and iron levels were measured using a Model ZEEnit 700 atomic absorption spectrometer (AAS) (Analytik Jena, Jena, Germany) that was equipped with a transversely heated graphite tube atomizer and a Zeeman effect background correction system (Bertuloso et al., 2015). Briefly, the samples were directly weighed on graphite platforms using an analytical microbalance and were introduced into the graphite tubes using a Model SSA 600 for automatic solid direct sampling. An iron hollow cathode lamp was employed as a light source (Analytik Jena, Jena, Germany), and the measurements were performed at an integrated absorbance of 248.3 nm. In addition, we utilized Pd (10,000 $\mu g\,mL^{-1}$ Merck) and $MgNO_3$ (1000 $\mu g\,mL^{-1}$ SCP Science) (10 μg Pd + 6 μg $MgNO_3$) as modifiers, which added to each measurement of a sample or standard, diluted in 0.2% (v/v) ultra-pure HNO_3 or water (Elga—Purelab, Marlow, UK). The results are expressed in $\mu g/g$.

To evaluate the tissue iron concentrations, spleen, liver, hypothalamus, ovary and uterus samples were collected and iron levels were assessed using inductively coupled plasma-optical emission spectrometry (ICP-OES) (Perkin Elmer 7000 DV) (Bressy et al., 2013). Briefly, tissue samples were digested with 30% of H_2O_2 (m/m) and ultra-pure HNO_3 (Elga—Purelab, Marlow, UK) using a microwave oven equipped with PTFE vessels (Multiwave 3000 microwave, Anton Paar, Graz, Austria). The analyses were performed with an ICP-OES, and argon gas (99,999% Air Liquid, RJ, Brazil) was employed in the determinations of ICP-OES for plasma generation, nebulization and auxiliary gas. The sample introduction system was composed of a cyclonic spray chamber and a Meinhard® nebulizer. The method's quality control section requirements were strictly applied to obtain an accurate quantification of iron in the tissue samples of this study. The ICP-OES operating conditions