HHS Public Access

Author manuscript

Exp Gerontol. Author manuscript; available in PMC 2015 August 28.

Published in final edited form as:

Exp Gerontol. 2009 September ; 44(9): 594–600. doi:10.1016/j.exger.2009.06.006.

Age-dependent and gender-specific changes in mouse tissue iron by strain

Paul Hahn[1,*], **Ying Song**[1], **Gui-shuang Ying**[1], **Xining He**[1], **John Beard**[2,†], and **Joshua L. Dunaief**[1]

[1]F. M. Kirby Center for Molecular Ophthalmology, Scheie Eye Institute, University of Pennsylvania, Philadelphia, PA 19104

[2]Department of Nutrition, College of Health and Human Development, Pennsylvania State University, University Park, PA 16802

Abstract

Iron is necessary for life but also a potent pro-oxidant implicated in the pathogenesis of age-related diseases. We sought to determine if iron levels change with age and by sex in various tissues from several commonly studied mouse strains. Brain, liver, heart, retina, and retinal pigment epithelium (RPE)/choroid were dissected from male and female mice of young adult (2–6 month old) and aged (16–19 month old) C57BL/6, DBA/2J, and BALB/c mice. Iron was quantified through a chromagen-based spectrophotometric method or through atomic absorption spectrophotometry for increased sensitivity. Brain, liver, and heart iron increased by 30–70% in aged vs. young adult groups of all strains, while retina and RPE/choroid iron had variable age-related changes. Significant gender differences were observed in BALB/c and DBA/2J strains. Males had as much as 2–3 fold more brain, RPE/choroid, and retinal iron, while females had as much as 2–3 fold more liver iron. There was no significant gender difference observed in heart iron. The different profiles of change between gender and among strains suggest that hormones and genetics influence iron regulation with aging. Future manipulation of iron levels in mice will test the role of iron in aging and disease, and the data reported herein will be essential in directing such manipulations.

Keywords

Iron; oxidative stress; aging; gender; strain; mouse model; age-related macular degeneration; retinal degeneration; neurodegenerative disease; hemochromatosis

Introduction

Accumulation of oxidative stress throughout life has long been implicated in the normal aging process, and the role of iron as a pro-oxidant important in aging has been proposed (Polla, 1999; Polla et al., 2003). Iron is an essential cofactor for life-sustaining biological

[*]To whom correspondence should be addressed: Paul Hahn, MD, PhD, F.M. Kirby Center for Molecular Ophthalmology, 303 Stellar-Chance Labs, 422 Curie Blvd, Philadelphia, PA 19104, phone 215-779-1710, fax 215-573-3918, paul.hahn@uphs.upenn.edu.
[†]Deceased

enzymes but can also generate toxic reactive oxygen species. Free ferrous iron participates in the Fenton reaction, catalyzing the conversion of hydrogen peroxide to the hydroxyl radical, a highly reactive free radical that can cause extensive oxidative damage to lipids, DNA, and proteins (Halliwell, 2001).

There is evidence that iron may accumulate with age in various tissues in both humans and animal models. Human total body iron stores, reflected by serum ferritin levels, increase with age but at different rates in men and women (Cook et al., 1976; Dallman et al., 1980). The brains of normal humans accumulate iron with age, shown in post mortem brains (Hallgren and Sourander, 1958) and in vivo by MRI analysis (Bartzokis et al., 1994), and the actual role of iron accumulation in brain pathobiology is an active area of investigation. In normal human retina, another neural tissue, iron levels measured by atomic absorption spectrophotometry (AAS) also increase with age (Hahn et al., 2006). Similarly, in various rodent animal models, age-related increases in iron are seen in kidney, liver, heart, and brain (Massie et al., 1983; Cook and Yu, 1998; Maynard et al., 2002; Unger et al., 2007). Iron accumulates with age in *Drosophila melanogaster* (Massie et al., 1985), and inhibition of iron absorption in *Drosophila* results in prolonged lifespan (Massie et al., 1993), suggesting that the mismanagement of iron may not be merely a consequence of aging but rather may be involved in the pathogenesis of normal aging processes.

Excess iron has been implicated in the development of age-related diseases. Although somewhat controversial, high iron stores may confer increased risk for heart disease (Sullivan, 1989; Salonen et al., 1992) and cancer (Stevens et al., 1988; Zacharski et al., 2008), and hyperferritinemia has been associated with diabetes (Dinneen et al., 1992). Abnormal iron accumulation has been demonstrated in the maculas of patients with age-related macular degeneration (AMD) (Hahn et al., 2003), in the substantia nigra of patients with Parkinson’s disease (Youdim et al., 1989), and in the senile plaques and neurofibrillary tangles of Alzheimer’s brains (Smith et al., 1997; Perry et al., 2002). Mice genetically altered to accumulate iron in their retinal pigment epithelium (RPE) and retina demonstrate an age-dependent AMD-like phenotype (Hahn et al., 2004; Hadziahmetovic et al., 2008), and pharmacologic or genetic chelation of iron protects against an MPTP-induced model of Parkinson’s disease (Halliwell, 2001; Kaur et al., 2003).

Iron accumulation with aging suggests there is a gradual shift in the balance between iron absorption and iron excretion. Iron balance in humans is normally regulated at the level of iron absorption, and the only well described mechanism of iron loss in humans is through menstruation (Sullivan, 1981). Menstruating females have lower levels of serum ferritin than males, and it has been hypothesized that this difference may contribute to increased longevity in females (Sullivan, 1981). There is emerging evidence that iron regulation may also be influenced by estrogen and other gender-specific hormones (Stuckey et al., 2006), and human females were recently identified as having lower levels of brain ferritin iron, possibly accounting for the lower risk of neurodegenerative diseases in females (Bartzokis et al., 2007).

Further understanding of the potential role of iron in aging and disease will be improved with the use of animal models to allow for the controlled manipulation of iron. However, an

understanding of the normal changes in iron levels with age and between genders is an important precursor to an understanding of the implications of any such manipulations. In this study, we provide a systematic investigation into the changes in iron levels that occur with age in males and females of various tissues of three commonly studied mouse strains. We demonstrate strain-specific differences in iron levels with age and by gender in C57BL/6, DBA/2J, and BALB/c mice.

Materials and Methods

Animals

Wild-type mice of various ages from three different strains – C57BL/6, DBA/2J, and BALB/c – were obtained from the National Institute of Aging (Bethesda, MD) rodent colonies. Mice were fed ad libitum from a standard NIH-31 growth diet consisting of 300.20 mg/kg iron. Mice from the same strain were housed in the same facilities under equivalent conditions, and all studied female mice were nulliparous. All procedures were approved by Institutional Animal Care and Use Committee of the University of Pennsylvania.

Complete organs including liver, heart, brain, and eyes were harvested immediately after sacrifice. An initial pilot study was performed comparing iron levels in PBS-perfused and non-perfused animals, and no difference in iron levels was detectable (data not shown). All mice included in the data analysis of this study were consequently non-perfused.

Liver, heart, and brain were rinsed in PBS, wet weights were recorded, and samples were stored at −80°C until iron quantification was carried out. Eyes were fixed in 4% paraformaldehyde before dissection for iron quantification.

Quantitative Iron Detection

Total non-heme iron levels in liver, heart, and brain were quantified using a modified bathophenanthroline-based spectrophotometric protocol originally described by Torrance and Bothwell (Torrance and Bothwell, 1968; Patel et al., 2002). Briefly, pre-weighed tissues frozen at −80°C were thawed at room temperature followed by digestion overnight at 65°C in 0.1% trichloroacetic acid/0.03M HCl. Periodically throughout digestion, the tissue-acid mixture was vigorously vortexed to facilitate digestion. Following digestion, samples were allowed to cool to room temperature and then centrifuged at room temperature. 20μl of the resulting supernatant was added to 1ml of chromagen reagent consisting of 2.25M sodium acetate (pretreated with Chelex 100 before vacuum filtration), 0.01% bathophenanthroline, and 0.1% thioglycolic acid. These mixtures were thoroughly vortexed and incubated at room temperature for 10 minutes before absorbances were read at 535nm. Iron levels measured in μg iron per gram tissue (wet weight) were determined by comparing absorbances of tissue-chromagen samples to serial dilutions of a certified iron standard (Sigma-Aldrich, Inc., St Louis, MO) prepared with the same chromagen reagent above.

Iron detection in ocular samples was performed by atomic absorption spectrophotometry as previously described (Erikson et al., 1997). Briefly, eyes enucleated and fixed in 4% PFA were dissected with complete removal of any periorbital muscle or fat, the anterior segment, and the ciliary body. Complete retina was then detached from the underlying tissue, taking

care to minimize disruption of the RPE. Samples of the retina and RPE/choroid were placed in separate tubes and dried for 5 days at room temperature. Iron in these tissues was measured by graphite furnace atomic absorption spectrophotometry (model 5100 AA, PerkinElmer, Boston, MA) using standard methods (Erikson et al., 1997). Because of the difficulty in accurately measuring the weights of these small tissues, ocular tissue iron levels were expressed in μg iron per whole retina or RPE/choroid sample. Quality control external and internal standards were included in every analytical run with a % CV < 3% within a run and <5% between runs.

Statistical analysis

For each strain, the effect of age, sex, and their interaction on the iron levels in various tissues was first assessed by two-way analyses of variance (ANOVA). If the interaction between age and sex was statistically significant ($p<0.05$) or marginally significant ($p<0.10$), the comparison of iron levels between male vs. female was made for each age group – young adult (2–6 months) and aged (16–19 months) – and between young adult vs. aged for each sex, based on the ANOVA including both main effects of age, sex, and their interaction; the Bonferroni procedure was used to adjust the multiple comparisons. If the interaction between age and sex was not statistically significant, the comparison of iron level was made for male vs. female and for young adult vs. aged groups, based on the ANOVA including only main effects of age and sex.

Because iron levels measured from both eyes of a mouse are correlated, the analysis of iron level data in retina and RPE should consider the inter-eye correlation. Ignoring the inter-eye correlation can underestimate the standard error of iron levels, leading to invalid statistical inferences. Thus, in the comparison of iron levels in retina and RPE/choroid, we used the generalized estimating equation (GEE) approach to adjust for the correlation between paired eyes of the same mouse (Zeger et al., 1988). Calculations were executed on computer using PROC GENMOD (SAS version 9.1; SAS, Cary, NC) and we specified an exchangeable working correlation structure to describe the correlation in iron levels between paired eyes of a mouse. Data from each mouse are identified to the computing algorithm by specifying a unique identification number for each mouse. For mice with iron levels from both eyes, the correlation between eyes is involved in the calculation of standard errors; while for mice with iron levels measured from only one eye, no correlation is considered in the calculation of standard errors.

For each tissue, the comparison of iron levels among different strains is also performed by using ANOVA, which includes the main effect of strain, age and sex. The post-hoc pairwise comparisons were performed when the overall difference among 3 strains was statistically significant ($p<0.05$). Two-sided $p<0.05$ was considered to be statistically significant. All statistical analyses were performed in SAS 9.1.

Results

Iron levels were compared among young adult (2–6 months) and aged (16–19 months) male and female mice of different strains. Among the C57BL/6 mice, 12 were 3–6 months old and 6 were 16 months old, yielding 12 adult and 6 aged samples of brain, liver, and heart

and 24 adult and 12 aged samples of retina and RPE/choroid (Table 1). Among BALB/c mice, 12 were 2–4 months old and 12 were 18–19 months old, yielding 12 adult and 12 aged samples of brain, liver, and heart and 19 adult and 20 aged samples of retina and RPE/choroid. Among the DBA/2J mice, 6 mice were 5 months old and 6 mice were 18 months old, yielding 6 adult and 6 aged samples of brain, liver, and heart and 11 adult and 11 aged samples of retina and RPE/choroid. No samples were lost during harvesting; odd numbers of ocular samples resulted from use of some samples for histologic and other analysis (not shown). There were no significant changes in the weights of any of the measured tissues (brain, liver, or heart) with age (not shown).

Strain-related differences

Comparison was first made between mean tissue iron levels of different strains by using analysis of variance, adjusted for the main effect of age and sex (Table 2). In brain, liver, retina, and RPE/choroid, we found statistically significant differences in iron levels up to 3–4 fold among strains. Pairwise comparisons revealed these differences to be significant among all strains except for DBA/2J and BALB/c livers. We did not detect any differences in heart iron levels among strains.

Age- and gender-related differences

For each strain, the independent effect of age and sex on iron levels of various tissues was assessed by two-way ANOVA. For most tissues, there was no significant interaction between age and sex, suggesting that age and sex act independent of each other in affecting iron levels. The exceptions were DBA/2J livers with a significant interaction between age and sex ($p=0.02$), BALB/c RPE/choroid also with a significant age-sex interaction ($p=0.01$), and BALB/c retinas with a marginally significant interaction ($p=0.08$); in these tissues, the difference in iron levels with aging may be different in male and female mice, and the difference in iron levels between gender may be different for young adult and aged mice, thus necessitating analysis of each age group by gender.

Brain—Comparison of iron levels in whole brains of young adult versus aged mice (Table 3) demonstrated statistically significant increases with age in all studied strains. C57BL/6 brains had a 32.5% increase in iron with age ($p=0.0001$), DBA/2J had a 44.4% increase ($p<0.0001$), and BALB/c had a 69.9% increase ($p<0.0001$).

Comparison of brain iron levels by gender demonstrated significant differences in DBA/2J and BALB/c mice. Male DBA/2J mice had 66.1% more iron than females ($p<0.0001$), while male BALB/c mice had 35.6% more iron than females ($p<0.0001$). There was no significant gender difference in iron levels of C57BL/6 mice ($p=0.56$).

Liver—Comparison of iron levels in the livers of young adult and aged mice (Table 4) demonstrated statistically significant increases with age in C57BL/6 and BALB/c aged mice. C57BL/6 livers had a 29.6% increase in iron with age ($p=0.0001$), and BALB/c had a 39.4% increase ($p<0.005$). Comparison of iron levels by sex demonstrated 61.9% more iron in female BALB/c females compared to males ($p=0.0002$); there was no significant gender difference in C57BL/6 liver iron ($p=0.25$).

DBA/2J liver iron had a significant interaction between age and sex (p=0.02). Male DBA/2J livers had a non-significant 32.1% increase with age in liver iron (p=0.96), while female DBA/2J livers had a significant 68.9% increase with age (p=0.0028). Young adult female DBA/2J livers had 91.6% more iron than young adult male livers (p=0.028), while aged female livers had 145.1% more iron than aged male livers (p<0.0001).

Heart—Comparison of iron levels in the hearts of young adult and aged mice (Table 5) demonstrated statistically significant increases with age in BALB/c and DBA/2J mice. BALB/c mice had a 55.9% increase in iron (p=0.007) and DBA/2J mice had a 42.3% increase (p=0.03). C57BL/6 hearts had a 14.1% increase in iron that was not statistically significant (p=0.21). There were no significant differences in heart iron levels between male vs. female C57BL/6 (p=0.59), BALB/c (p=0.40), or DBA/2J mice (p=0.38).

Retina—Comparison of iron levels in the retinas of adult and aged mice (Table 6) demonstrated a significant 38.3% decrease with age in C57BL/6 mice (p=0.008) but a significant 47.7% increase in DBA/2J mice (p=0.02). Comparison of retinal iron levels between males and females demonstrated no difference in C57BL/6 mice (p=0.52) but 37.0% more iron in male DBA/2J retinas than female DBA/2J retinas (p=0.046).

BALB/c retinas had a marginally significant interaction between age and sex (p=0.08). Male BALB/c retinal iron was unchanged with age (p=1.00), and female BALB/c retinas had a non-significant 24.0% decrease in iron with age (p=0.12). Male young adult BALB/c retinas had non-significant 20.0% more iron than female young adult retinas (p=0.30), while male aged retinas had significant 63.2% more iron than female aged BALB/c retinas (p<0.0001).

Retinal pigment epithelium/choroid—Comparison of iron levels in the RPE/choroid of young adult and aged mice (Table 7) demonstrated a significant 42.7% increase with age in C57BL/6 mice (p=0.01). DBA/2J mice had a 13.6% increase in iron that was not statistically significant (p=0.31). Comparison of RPE/choroid iron levels by gender revealed 75.9% more iron in RPE/choroid of male DBA/2J mice compared to that of female mice (p=0.008). There were no detectable gender differences in RPE/choroid of C57BL/6 mice (p=0.27).

BALB/c RPE/choroid had a significant interaction between age and sex (p=0.01). Male BALB/c RPE/choroid had a significant 48.9% increase with age (p<0.0001), while the 22.7% increase in female RPE/choroid iron with age was not significant (p=0.76). Male BALB/c mice had 100.0% more iron than females in the young adult group (p<0.0001) and 142.6% more iron in the aged group (p<0.0001).

Discussion

Iron has been increasingly implicated in the pathogenesis of aging and disease, and the use of animal models will be important in furthering an understanding of this putative role for iron. In this study, we systematically compared iron levels with age and gender among three commonly studied mouse strains – C57BL/6, DBA/2J, and BALB/c – to better understand normal mechanisms of iron regulation and to provide a framework for guiding future mouse-

based investigations altering normal iron levels. We demonstrate that there are age-related, gender-related, and strain-related differences in iron levels among various tissues.

Iron accumulation has been implicated in the pathogenesis of several age-related diseases, and understanding the mechanisms of normal increases in iron with age will be important in elucidating mechanisms of putative iron-related changes in disease. We compared mice 2–6 months of age to mice 16–19 months of age. Studies on mean longevity of various strains of mice have demonstrated that C57BL/6 mice are relatively longlived, with a mean lifespan of approximately 27–28 months. DBA/2J mice, on the other hand, are shorter lived with a mean lifespan of approximately 23–24 months (Goodrick, 1975). The same study showed that male BALB/c mice are similarly short lived, with a mean lifespan of 21–22 months, while female BALB/c mice are long lived, with a mean lifespan of 27 months. In this study, mice 2–6 months were selected as fully developed “young adult” mice, while mice 16–19 months were chosen to represent healthy “aged” mice before the onset of senescent decline. These ages likely represent different points in the relative lifespans of the different strains, which may account for some of the observed strain-specific differences in iron accumulation with age.

To confirm that any observed age-related changes in tissue iron represent true alterations of iron and not simply an artifact of decreasing tissue mass with age, we analyzed the weights of brain, liver, and heart removed in toto from both adult and aged mice. Analysis of RPE/choroid and retina samples was performed per whole tissue, obviating any consideration of this potential artifact. There were no significant differences between the weights of adult and aged tissues from any strain, suggesting that the measured increases in iron represent age-dependent accumulation of iron.

Our finding of up to 70% increased iron levels with age in various tissues across all strains is consistent with previous studies. C57BL/6 mice demonstrate age-dependent iron increases in liver, heart, brain, and kidney (Massie et al., 1983). Male Fischer 344 rats accumulate iron in kidney, liver, and brain (Cook and Yu, 1998). Age-related increases in iron levels have also been found in blood, liver, kidney, heart, brain, lung, and spleen of female B10BR mice (Morita et al., 1994). C57BL6/DBA and C57BL6/SJL hybrid strains exhibit increases in brain iron levels (Maynard et al., 2002). Within the eye, iron increases with age in male Brown Norway rat retinas (Chen et al., 2008) and in normal human male and female retinas (Hahn et al., 2006).

Our current study underscores these results and adds information on iron levels in additional strains and tissues, systematically comparing these age-related increases among various strains and by gender in diverse tissues including ocular structures. While increased iron with age may merely be a by product of normal aging processes or disease, iron may itself be involved in the pathogenesis of aging and/or age-related diseases. Strain-related differences in iron levels may contribute to known differences in lifespan among strains and warrants further exploration. Investigations are currently underway to determine if alteration of iron levels in mice might affect the development of age-related pathology or even alter lifespan, and the results from this study have been critical in establishing a systematic basis to understand iron accumulation as well as guide selection of studied mouse strains.

In addition to demonstrating increased iron levels with age, our study highlights differences in iron levels with gender. Previous studies have demonstrated that human females of all ages generally have less serum ferritin than males (Zacharski et al., 2000). Human females have less brain ferritin iron than males (Bartzokis et al., 2007), and mixed BL6/DBA females have less brain iron than males (Maynard et al., 2006), similar to the gender differences in brain iron observed in our DBA/2J and BALB/c mice. We have previously shown that human females have more retinal iron than males (Hahn et al., 2006), in contrast to the lower levels of retinal iron observed in DBA/2J and BALB/c female mice. Previous studies on the effects of iron overload on C57BL/6 and DBA/2J mice detected less iron in the liver and spleen of males compared to females of DBA/2J mice, consistent with our studies, and of C57BL/6 mice, in contrast to the absence of any gender differences detectable in our study (Unger et al., 2007).

We were surprised not to find any effect of gender on iron levels in C57BL/6 mice. In a study investigating brain metal levels in hybrid BL6/DBA and BL6/SJL mice, a gender difference in brain iron levels was observed in BL6/DBA but not BL6/SJL mice (Maynard et al., 2006). This gender difference was attributed to different age-related changes in iron levels, as there was no significant gender difference noted in young, <3 month old BL6/DBA mice (Maynard et al., 2006). C57BL/6 mice are the longest lived among the three strains included in this study, with a mean lifespan of 27–28 months, and it is similarly possible that our 16 month old mice were not old enough to demonstrate detectable gender differences in iron. Alternatively, our study may not have been adequately powered to detect subtle changes in iron levels. Further investigation into gender differences in C57BL/6 mice should include larger numbers of mice of more advanced age.

Gender-related differences in iron levels of up to 2–3 fold in our current study suggest that there may be hormonal mechanisms of iron regulation. We demonstrate that in DBA/2J livers, BALB/c retinas, and BALB/c RPE/choroid, males and females accumulate iron with age at different rates, consistent with similar observations in brain iron of different mouse strains (Maynard et al., 2006). The hormonal basis for these differences in iron levels should be investigated, and future studies on iron levels need to control for gender. This gender difference may account for gender-related associations of the pathogenesis of neurodegenerative and other diseases and may be important for designing gender-targeted treatment options.

Our present study is the first, in our knowledge, to systematically compare iron levels in multiple tissues among three commonly studied pure inbred strains. Strain specific differences in iron levels of individual tissues have previously been observed. We have shown that mice fed an iron-supplemented diet showed increased spleen iron accumulation in DBA females compared to C57BL/6 females (Unger et al., 2007). In another study with hybrid mouse strains, Maynard et al demonstrated different brain iron levels in C57BL6/SJL compared to C57BL6/DBA mice (Maynard et al., 2006). In this study, we analyze C57BL/6, DBA/2J, and BALB/c strains, which originated independently in North America and have been maintained as pure inbred colonies since the early twentieth century (Staats, 1985). In a study mapping genetic profiles of 27 inbred strains, C57BL/6, DBA/2J, and BALB/c are identified as genetically distinct, with C57BL/6 being the most distinct of these strains

(Taylor, 1972). We identify these distinct strains as having unique profiles of iron levels and changes in various tissues.

Identification of these strain specific differences will be important in guiding future iron-based investigations. A particular interest of our laboratory is age-related macular degeneration, and we have proposed that increased iron in the retina and RPE may be important in its pathogenesis. We have identified DBA/2J mice as having the largest increases in retinal iron, while C57BL/6 have the largest increases in RPE/choroid iron, and our study identifies these strains as appropriate initial models to test manipulations to decrease retina or RPE/choroid iron levels, studies which are currently underway. Our comparisons in other tissues should similarly help guide iron-based studies in brain, heart, or liver. These strain-specific differences are likely based on genetic differences among different strains, and our identification of differences in iron levels among pure inbred strains – C57BL/6, BALB/c, and DBA/2J – should help to identify these genetic influences, which we are currently investigating. Identification of the basis for these murine strain specific differences may also help identify differences in iron regulation among humans, which may account for variability in neurodegenerations or other potential iron-related diseases and may additionally be important in directing targeted therapies.

There is evidence that diurnal cycle influences iron homeostasis (Unger et al., 2009). While all mice were sacrificed during daytime hours, between 9am to 5pm, we did not standardize time of sacrifice. It is possible that observed variations in iron levels may be influenced by diurnal fluctuation at time of sacrifice, and future studies investigating iron levels should standardize time of sacrifice.

In assaying for iron, we used a well-established bathophenanthroline-based chromatographic protocol for the larger heart, brain, and liver samples, and we used atomic absorption spectrophotometry for increased sensitivity in the smaller retina and RPE/choroid samples. This chromatographic protocol has been previously shown to measure non-heme iron only (Grundy et al., 2004), which is useful to eliminate any variable contributions by intravascular iron from blood, particularly in richly perfused tissues such as liver. Our measurement of non-heme iron only likely explains the absence of any difference in measured iron levels in perfused and non-perfused animals (not shown). While non-heme iron is likely important in contributing to iron-related oxidative stress, it may additionally be of interest in the future to measure changes in intracellular heme-associated iron, as in mitochondrial cytochromes, which may also have the potential to induce oxidative stress.

Atomic absorption spectrophotometry, on the other hand, measures both heme and non-heme iron. Initial pilot experiments were performed analyzing the effects of perfusion prior to sacrifice. We did not detect any significant difference in any of our samples with and without perfusion (results not shown), and all animals included in this study were therefore non-perfused. This pilot study suggests that intravascular iron does not significantly contribute to our non-heme (in liver, brain, and heart) and total iron (in retina and RPE/choroid) measurements.

Interestingly, DBA/2J eyes are unique among these studied strains in that they have a pigmentary dispersion phenotype in which intraocular pigment is continually dispersed (Anderson et al., 2006). It is unclear if this dispersed pigment contains iron and/or is deposited on the retina. It is possible, however, that this progressive release of pigment may contribute to age-dependent increases in retinal iron. DBA/2J eyes also have increased intraocular pressure and age-dependent loss of retinal ganglion cells, making them a model of glaucoma (Anderson et al., 2006). Since changes in iron homeostasis have been detected in human glaucoma (Farkas et al., 2004), it is possible that similar mechanisms cause iron dysregulation in some human glaucoma retinas and DBA/2J retinas.

Conclusions

Iron has been increasingly implicated in aging and disease, and the use of animal models will be pivotal in understanding this role for iron. We provide the first systematic comparison of iron levels in three pure inbred mouse strains by age and gender and demonstrate that iron levels generally increase with age in various mouse tissues of different strains. Interestingly, tissue iron levels and changes in iron levels differ among various strains and between genders, suggesting that there are gender and strain-specific influences on iron regulation. Genetic studies pursuing the basis of some of these strain-dependent differences in iron homeostasis are underway (Unger et al., 2007). This study provides a baseline profile for iron levels in various tissues and in various strains, which will be important in directing future mouse studies involving manipulations of normal iron levels.

Acknowledgments

This work was supported by the Dennis Jahnigen Award from the American Geriatrics Society, the F.M. Kirby Foundation, and the Paul and Evanina Bell Mackall Foundation Trust. John Beard passed away during preparation of this manuscript. We are grateful for his significant contributions to this work and for his friendship.

REFERENCES

Anderson MG, Libby RT, Mao M, Cosma IM, Wilson LA, Smith RS, John SW. Genetic context determines susceptibility to intraocular pressure elevation in a mouse pigmentary glaucoma. BMC Biol. 2006; 4:20. [PubMed: 16827931]

Bartzokis G, Mintz J, Sultzer D, Marx P, Herzberg JS, Phelan CK, Marder SR. In vivo MR evaluation of age-related increases in brain iron. AJNR Am J Neuroradiol. 1994; 15(6):1129–1138. [PubMed: 8073983]

Bartzokis G, Tishler TA, Lu PH, Villablanca P, Altshuler LL, Carter M, Huang D, Edwards N, Mintz J. Brain ferritin iron may influence age- and gender-related risks of neurodegeneration. Neurobiol Aging. 2007; 28(3):414–423. [PubMed: 16563566]

Chen H, Liu B, Lukas TJ, Suyeoka G, Wu G, Neufeld AH. Changes in iron-regulatory proteins in the aged rodent neural retina. Neurobiol Aging. 2008

Cook CI, Yu BP. Iron accumulation in aging: modulation by dietary restriction. Mech Ageing Dev. 1998; 102(1):1–13. [PubMed: 9663787]

Cook JD, Finch CA, Smith NJ. Evaluation of the iron status of a population. Blood. 1976; 48(3):449–455. [PubMed: 953365]

Dallman PR, Siimes MA, Stekel A. Iron deficiency in infancy and childhood. Am J Clin Nutr. 1980; 33(1):86–118. [PubMed: 6986756]

Dinneen SF, O'Mahony MS, O'Brien T, Cronin CC, Murray DM, O'Sullivan DJ. Serum ferritin in newly diagnosed and poorly controlled diabetes mellitus. Ir J Med Sci. 1992; 161(11):636–638. [PubMed: 1478845]

Erikson KM, Pinero DJ, Connor JR, Beard JL. Regional brain iron, ferritin and transferrin concentrations during iron deficiency and iron repletion in developing rats. J Nutr. 1997; 127(10): 2030–2038. [PubMed: 9311961]

Farkas RH, Chowers I, Hackam AS, Kageyama M, Nickells RW, Otteson DC, Duh EJ, Wang C, Valenta DF, Gunatilaka TL, Pease ME, Quigley HA, Zack DJ. Increased expression of iron-regulating genes in monkey and human glaucoma. Invest Ophthalmol Vis Sci. 2004; 45(5):1410–1417. [PubMed: 15111596]

Goodrick CL. Life-span and the inheritance of longevity of inbred mice. J Gerontol. 1975; 30(3):257–263. [PubMed: 1120887]

Grundy MA, Gorman N, Sinclair PR, Chorney MJ, Gerhard GS. High-throughput non-heme iron assay for animal tissues. J Biochem Biophys Methods. 2004; 59(2):195–200. [PubMed: 15163531]

Hadziahmetovic M, Dentchev T, Song Y, Haddad N, He X, Hahn P, Pratico D, Wen R, Harris ZL, Lambris JD, Beard J, Dunaief JL. Ceruloplasmin/hephaestin knockout mice model morphologic and molecular features of AMD. Invest Ophthalmol Vis Sci. 2008; 49(6):2728–2736. [PubMed: 18326691]

Hahn P, Milam AH, Dunaief JL. Maculas affected by age-related macular degeneration contain increased chelatable iron in the retinal pigment epithelium and Bruch's membrane. Arch Ophthalmol. 2003; 121(8):1099–1105. [PubMed: 12912686]

Hahn P, Qian Y, Dentchev T, Chen L, Beard J, Harris ZL, Dunaief JL. Disruption of ceruloplasmin and hephaestin in mice causes retinal iron overload and retinal degeneration with features of age-related macular degeneration. Proc Natl Acad Sci U.S.A. 2004; 101(38):13850–13855. [PubMed: 15365174]

Hahn P, Ying GS, Beard J, Dunaief JL. Iron levels in human retina: sex difference and increase with age. Neuroreport. 2006; 17(17):1803–1806. [PubMed: 17164668]

Hallgren B, Sourander P. The effect of age on the non-haemin iron in the human brain. J Neurochem. 1958; 3(1):41–51. [PubMed: 13611557]

Halliwell B. Role of free radicals in the neurodegenerative diseases: therapeutic implications for antioxidant treatment. Drugs Aging. 2001; 18(9):685–716. [PubMed: 11599635]

Kaur D, Yantiri F, Rajagopalan S, Kumar J, Mo JQ, Boonplueang R, Viswanath V, Jacobs R, Yang L, Beal MF, DiMonte D, Volitaskis I, Ellerby L, Cherny RA, Bush AI, Andersen JK. Genetic or pharmacological iron chelation prevents MPTP-induced neurotoxicity in vivo: a novel therapy for Parkinson's disease. Neuron. 2003; 37(6):899–909. [PubMed: 12670420]

Massie HR, Aiello VR, Banziger V. Iron accumulation and lipid peroxidation in aging C57BL/6J mice. Exp Gerontol. 1983; 18(4):277–285. [PubMed: 6667718]

Massie HR, Aiello VR, Williams TR. Iron accumulation during development and ageing of Drosophila. Mech Ageing Dev. 1985; 29(2):215–220. [PubMed: 3919221]

Massie HR, Aiello VR, Williams TR. Inhibition of iron absorption prolongs the life span of Drosophila. Mech Ageing Dev. 1993; 67(3):227–237. [PubMed: 8326745]

Maynard CJ, Cappai R, Volitakis I, Cherny RA, Masters CL, Li QX, Bush AI. Gender and genetic background effects on brain metal levels in APP transgenic and normal mice: implications for Alzheimer beta-amyloid pathology. J Inorg Biochem. 2006; 100(5–6):952–962. [PubMed: 16574231]

Maynard CJ, Cappai R, Volitakis I, Cherny RA, White AR, Beyreuther K, Masters CL, Bush AI, Li QX. Overexpression of Alzheimer's disease amyloid-beta opposes the age-dependent elevations of brain copper and iron. J Biol Chem. 2002; 277(47):44670–44676. [PubMed: 12215434]

Morita A, Kimura M, Itokawa Y. The effect of aging on the mineral status of female mice. Biol Trace Elem Res. 1994; 42(2):165–177. [PubMed: 7981006]

Patel BN, Dunn RJ, Jeong SY, Zhu Q, Julien JP, David S. Ceruloplasmin regulates iron levels in the CNS and prevents free radical injury. J Neurosci. 2002; 22(15):6578–6586. [PubMed: 12151537]

Perry G, Sayre LM, Atwood CS, Castellani RJ, Cash AD, Rottkamp CA, Smith MA. The role of iron and copper in the aetiology of neurodegenerative disorders: therapeutic implications. CNS Drugs. 2002; 16(5):339–352. [PubMed: 11994023]

Polla AS, Polla LL, Polla BS. Iron as the malignant spirit in successful ageing. Ageing Res Rev. 2003; 2(1):25–37. [PubMed: 12437994]

Polla BS. Therapy by taking away: the case of iron. Biochem Pharmacol. 1999; 57(12):1345–1349. [PubMed: 10353254]

Salonen JT, Nyyssonen K, Korpela H, Tuomilehto J, Seppanen R, Salonen R. High stored iron levels are associated with excess risk of myocardial infarction in eastern Finnish men. Circulation. 1992; 86(3):803–811. [PubMed: 1516192]

Smith MA, Harris PL, Sayre LM, Perry G. Iron accumulation in Alzheimer disease is a source of redox-generated free radicals. Proc Natl Acad Sci U.S.A. 1997; 94(18):9866–9868. [PubMed: 9275217]

Staats J. Standardized Nomenclature for Inbred Strains of Mice: eighth listing. Cancer Res. 1985; 45(3):945–977. [PubMed: 3971387]

Stevens RG, Jones DY, Micozzi MS, Taylor PR. Body iron stores and the risk of cancer. N Engl J Med. 1988; 319(16):1047–1052. [PubMed: 3173433]

Stuckey R, Aldridge T, Lim FL, Moore DJ, Tinwell H, Doherty N, Davies R, Smith AG, Kimber I, Ashby J, Orphanides G, Moggs JG. Induction of iron homeostasis genes during estrogen-induced uterine growth and differentiation. Mol Cell Endocrinol. 2006; 253(1–2):22–29. [PubMed: 16684588]

Sullivan JL. Iron and the sex difference in heart disease risk. Lancet. 1981; 1(8233):1293–1294. [PubMed: 6112609]

Sullivan JL. The iron paradigm of ischemic heart disease. Am Heart J. 1989; 117(5):1177–1188. [PubMed: 2653014]

Taylor BA. Genetic relationships between inbred strains of mice. J Hered. 1972; 63(2):83–86. [PubMed: 5031317]

Torrance JD, Bothwell TH. A simple technique for measuring storage iron concentrations in formalinised liver samples. S Afr J Med Sci. 1968; 33(1):9–11. [PubMed: 5676884]

Unger EL, Beard JL, Jones BC. Iron regulation in C57BLI6 and DBA/2 mice subjected to iron overload. Nutr Neurosci. 2007; 10(1–2):89–95. [PubMed: 17539487]

Unger EL, Earley CJ, Beard JL. Diurnal cycle influences peripheral and brain iron levels in mice. J Appl Physiol. 2009; 106(1):187–193. [PubMed: 18988764]

Youdim MB, Ben-Shachar D, Riederer P. Is Parkinson's disease a progressive siderosis of substantia nigra resulting in iron and melanin induced neurodegeneration? Acta Neurol Scand Suppl. 1989; 126:47–54. [PubMed: 2618593]

Zacharski LR, Chow BK, Howes PS, Shamayeva G, Baron JA, Dalman RL, Malenka DJ, Ozaki CK, Lavori PW. Decreased cancer risk after iron reduction in patients with peripheral arterial disease: results from a randomized trial. J Natl Cancer Inst. 2008; 100(14):996–1002. [PubMed: 18612130]

Zacharski LR, Ornstein DL, Woloshin S, Schwartz LM. Association of age, sex, and race with body iron stores in adults: analysis of NHANES III data. Am Heart J. 2000; 140(1):98–104. [PubMed: 10874269]

Zeger SL, Liang KY, Albert PS. Models for longitudinal data: a generalized estimating equation approach. Biometrics. 1988; 44(4):1049–1060. [PubMed: 3233245]

Table 1

Summary table of studied samples

			Brain (n)	Liver (n)	Heart (n)	Retina (n)	RPE/choroid (n)
C57BL/6	Adult (3–6mos, n=12)	Male (n=6)	6	6	6	12	12
		Female (n=6)	6	6	6	12	12
	Aged (16mos, n=6)	Male (n=3)	3	3	3	6	6
		Female (n=3)	3	3	3	6	6
BALB/c	Adult (2–4mos, n=12)	Male (n=6)	6	6	6	9	9
		Female (n=6)	6	6	6	10	10
	Aged (18–19mos, n=12)	Male (n=6)	6	6	6	10	10
		Female (n=6)	6	6	6	10	10
DBA/2J	Adult (5mos, n=6)	Male (n=3)	3	3	3	5	5
		Female (n=3)	3	3	3	6	6
	Aged (18mos, n=6)	Male (n=3)	3	3	3	5	5
		Female (n=3)	3	3	3	6	6

Table 2

Comparison of adjusted mean tissue iron levels among strains

	Mean (SE)				
	Brain (μg/g)	**Liver (μg/g)**	**Heart (μg/g)**	**Retina (μg/sample)**	**RPE/choroid (μg/sample)**
C57BL/6	19.1 (0.78)	65.8 (14.0)	62.7 (4.45)	0.04 (0.003)	0.12 (0.008)
BALB/c	23.8 (0.67)	210 (11.9)	66.4 (3.78)	0.03 (0.001)	0.08 (0.004)
DBA/2J	12.2 (0.94)	245 (16.8)	54.1 (5.35)	0.54 (0.005)	0.15 (0.01)
Overall P-value	<0.0001	<0.0001	0.18	0.006	0.002
P-value for each pairwise comparison					
BALB/c vs. C57BL/6	<0.0001	<0.0001		0.0003	<0.0001
DBA/2J vs. C57BL/6	<0.0001	<0.0001		0.003	0.005
DBA/2J vs. BALB/c	<0.0001	0.10		<0.0001	<0.0001

Table 3

Comparison of mean brain iron levels (μg/g – wet weight) in different strains with age and gender

BRAIN	≤ 6 mo	≥16 mo		Male	Female	
	Mean (SE)	Mean (SE)	p-value	Mean (SE)	Mean (SE)	p-value
C57BL/6	16.0 (0.60)	21.2 (0.85)	0.0001	18.3 (0.71)	18.9 (0.71)	0.56
BALB/c	17.6 (0.64)	29.9 (0.64)	<0.0001	27.4 (0.64)	20.2 (0.64)	<0.0001
DBA/2J	9.97 (0.39)	14.4 (0.39)	<0.0001	15.2 (0.39)	9.15 (0.39)	<0.0001

Table 4

Comparison of mean liver iron levels (μg/g – wet weight) in different strains with age and gender

LIVER	≤ 6 mo	≥16 mo		Male	Female	
	Mean (SE)	Mean (SE)	p-value	Mean (SE)	Mean (SE)	p-value
C57BL/6	50.6 (1.66)	65.6 (2.35)	0.0001	56.5 (1.98)	59.8 (1.98)	0.25
BALB/c	175 (15.7)	244 (15.7)	0.005	160 (15.7)	259 (15.7)	0.0002
DBA/2J						
≤ 6 mo				131 (23.2)	251 (23.2)	0.028
≥ 16 mo				173 (23.2)	424 (23.2)	<0.0001
p-value				0.96	0.0028	

Table 5

Comparison of mean heart iron levels (μg/g – wet weight) in different strains with age and gender

HEART	≤ 6 mo	≥ 16 mo		Male	Female	
	Mean (SE)	Mean (SE)	p-value	Mean (SE)	Mean (SE)	p-value
C57BL/6	56.7 (3.49)	64.7 (4.94)	0.21	59.1 (4.16)	62.3 (4.16)	0.59
BALB/c	51.9 (6.80)	80.9 (6.80)	0.007	70.5 (6.80)	62.3 (6.80)	0.40
DBA/2J	44.7 (5.32)	63.6 (5.32)	0.03	57.6 (5.32)	50.7 (5.32)	0.38

Table 6

Comparison of mean retinal iron levels (μg/sample) in different strains with age and gender

RETINA	≤ 6 mo	≥16 mo		Male	Female	
	Mean (SE)	Mean (SE)	p-value	Mean (SE)	Mean (SE)	p-value
C57BL/6	0.047 (0.004)	0.029 (0.002)	0.008	0.036 (0.003)	0.040 (0.004)	0.52
DBA/2J	0.044 (0.004)	0.065 (0.006)	0.02	0.063 (0.006)	0.046 (0.003)	0.046
BALB/c						
≤ 6 mo				0.030 (0.002)	0.025 (0.002)	0.30
≥ 16 mo				0.031 (0.002)	0.019 (0.002)	<0.0001
p-value				1.00	0.12	

Table 7

Comparison of mean RPE/choroid iron levels (μg/sample) in different strains with age and gender

RPE/CHOROID	≤ 6 mo	≥16 mo		Male	Female	
	Mean (SE)	Mean (SE)	p-value	Mean (SE)	Mean (SE)	p-value
C57BL/6	0.103 (0.009)	0.147 (0.008)	0.01	0.132 (0.010)	0.118 (0.007)	0.27
DBA/2J	0.140 (0.005)	0.159 (0.018)	0.31	0.190 (0.019)	0.108 (0.003)	0.008
BALB/c						
≤ 6 mo				0.088 (0.005)	0.044 (0.006)	<0.0001
≥ 16 mo				0.131 (0.008)	0.054 (0.005)	<0.0001
p-value				<0.0001	0.76	

Table 8

Summary of age and gender effects on tissue iron levels by strain

	Brain	Liver	Heart	Retina	RPE/choroid
Age effect (%change, aged vs adult)					
C57BL/6	32.5%	29.6%	NS	−38.3%	42.7%
BALB/c	69.9%	39.4%	55.9%	NS	Male: 48.9%; Female: 22.7% (NS)
DBA/2J	44.4%	Male: 32.1% (NS); Female: 68.9%	42.3%	47.7%	NS
Sex effect (%difference, male vs female)					
C57BL/6	NS	NS	NS	NS	NS
BALB/c	35.6%	−38.2%	NS	Young: 20.0% (NS); Aged: 63.2%	Young: 100% Aged: 143%
DBA/2J	66.1%	Young: −47.8%; Aged: −59.2%	NS	37.0%	75.9%

NS: Not statistically significant.