

# Iron levels in human retina: sex difference and increase with age

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Oxidative stress is believed to be important in physiological aging and age-related diseases. Iron is a potent pro-oxidant implicated in several age-related diseases. While serum ferritin, as an estimate of body stores of iron, has been shown to increase with age, few studies have directly addressed the effect of age on human neural tissue iron levels. We used atomic absorption spectrophotometry to assess quantitatively iron levels within the eye structures, retina

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and retinal pigment epithelium/choroid of normal human eyes of various ages and of both sexes. We found that retinal iron increases with age, similar to serum ferritin levels. Women had more retinal iron than men at all ages, suggesting that there may be gender-specific influences on iron regulation. *NeuroReport* 17:1803–1806  
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## Introduction

Iron is both an essential cofactor for life-sustaining enzymes and also a potent generator of damaging free radicals. While complications of iron deficiency have long been recognized, adverse consequences of physiological iron overload have only more recently been investigated. Iron accumulation and the resulting oxidative stress in tissues have been implicated in the pathogenesis of several age-related human diseases, including heart disease [1,2], cancer [3], diabetes [4], and neurodegenerations such as Alzheimer's disease, Parkinson's disease, and age-related macular degeneration [5,6]. Additionally, excess iron has been associated with accelerated senescence and decreased lifespan in *Drosophila* [7]. Accumulation of body iron stores occurs with age [8,9] and may be important in both aging and age-related diseases.

Previous studies on human body iron stores have examined serum ferritin levels or other similar surrogates to estimate iron stores. Serum ferritin is an acute-phase reactant, which increases in states of inflammation and disease [10], and thus may not be an accurate representation of tissue levels of iron. A paucity of studies exist assaying directly for iron levels in human tissue, particularly in tissues other than the liver, heart, and spleen and from individuals from various age groups [11]. We present herein the first report demonstrating age-dependent accumulation of iron stores measured directly in post-mortem human eye samples using atomic absorption spectrophotometry.

## Methods

### Source of tissue and population profile

Post-mortem eyes were obtained through the National Disease Research Interchange, Philadelphia, Pennsylvania, USA. The pathology reports generally provided the patient's age, sex, brief ocular and medical histories, cause of death, and post-mortem interval.

Twenty-nine healthy eyes from 17 Caucasian donors were studied (Table 1). These eyes consisted of 14 male eyes (eight donors) and 15 female eyes (nine donors) divided into 'younger' ( $\leq 35$  years old) and 'older' ( $\geq 65$  years old) groups. The younger male group consisted of five eyes (three donors) with a mean age of 24.8 years (range: 17–35 years) and a mean post-mortem interval of 7.9 h (range: 3–13 h). The older male group consisted of nine eyes (five donors) with a mean age of 74.1 years (range: 65–83 years) and a mean post-mortem interval of 5.6 h (range: 2.5–10 h). The younger female group consisted of eight eyes (four donors) with a mean age of 17.8 years (range: 4–32 years); two of the younger female eyes (one donor) did not have a documented post-mortem interval, and the mean post-mortem interval for the other six eyes was 9.3 h (range: 3–18 h). The older female group consisted of seven eyes (five donors) with a mean age of 73.1 years (range: 65–88 years) and a mean post-mortem interval of 5.4 h (range: 2–8 h). Causes of death included trauma, cancers, cardiac abnormalities (e.g. myocardial infarction, arrhythmias, congestive heart failure), stroke, respiratory failure, and aortic

**Table 1** Characteristics of study subjects ( $n=17$  subjects, 29 eyes)

Subject characteristic	Eyes ( $n=29$ )	Subjects ( $n=17$ )
Age (years)		
Mean (SD)	50 (28)	53 (29)
Median (range)	65 (4–88)	65 (4–88)
Sample size $n$ (%)		
Younger ( $\leq 35$ years)	13 (44.8)	7 (41.2)
Older ( $\geq 65$ years)	16 (55.2)	10 (58.8)
Male	14 (48.3)	8 (47.1)
Female	15 (51.7)	9 (52.3)

aneurysm rupture. Two patients with chronic conditions that might alter tissue iron levels, gastrointestinal bleed, and failure to thrive were excluded. The histopathological studies followed the tenets of the Declaration of Helsinki, and informed consent was obtained from all eye donors before death. Approval for research on human post-mortem donor eyes was obtained from the University of Pennsylvania, Philadelphia, Pennsylvania, USA.

### Dissection

The anterior segments of fixed eyes were dissected away using a fresh razor blade through the pars plana. The optic nerve and the macula were identified under a Nikon TE-300 dissecting microscope (Nikon, Tokyo, Japan), and a 6 mm corneal trephine (Katena Products, Inc., Denville, New Jersey, USA) was used to separate a wafer containing the macular retina and the underlying retinal pigment epithelium (RPE), choroids, and sclera from the remaining eyecup. Any overlying vitreous matter was dissected away from this wafer.

The macular retina was then detached from the underlying wafer using nontoothed forceps, taking care to minimize disruption of the RPE. The separated retina was examined under the microscope for the presence of any adherent RPE, which, if present, was removed. The RPE and choroid were then dissected from the underlying sclera, and the remaining sclera was scraped with curved forceps to remove any remaining loosely adherent choroid, which was pooled with the RPE/choroid sample.

### Quantitative iron detection

Samples of the retina and RPE/choroid were placed in separate Eppendorf tubes and dried for 5 days at room temperature. Iron in these tissues was measured in triplicate by graphite furnace atomic absorption spectrophotometry (model 5100 AA; PerkinElmer, Boston, Massachusetts, USA) using standard methods [12]. Both internal and external quality control standards were included in the analysis. The coefficient of variance was less than 3% within a run.

### Data analysis

The association of age with iron levels in the retina and RPE/choroid was assessed using linear regression analysis by treating age as a continuous variable, and also by comparing mean iron levels between younger ( $\leq 35$  years) and older ( $\geq 65$  years) groups, adjusted by sex. The mean iron levels between male and female samples were also compared with the adjustment of age (as a continuous variable). As iron levels in some cases were measured from both eyes of the same individual, eye-specific analyses were

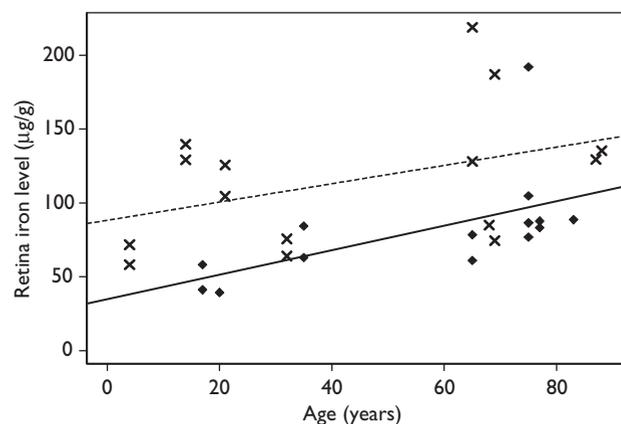
performed in which data from both eyes were included in the regression analysis, with the correlation between paired eyes from the same individual being adjusted by generalized estimating equations [13,14]. The mean, standard error of the mean (SEM), and  $P$  values for the comparison of means between groups (younger vs. older; men vs. women) were obtained from regression analysis using generalized estimating equations. To examine the similarity of iron levels of paired eyes from the same individual, the difference in the iron levels between paired eyes of same individual was calculated, and the intereye difference in iron was tested by the nonparametric Wilcoxon's signed-rank test owing to the skewed distribution.  $P < 0.05$  was considered to be statistically significant.

### Results

Measured by atomic absorption spectrophotometry, retinal samples (mean:  $102.3 \mu\text{g/g}$ ; range:  $41.1$ – $218.8 \mu\text{g/g}$ ) had significantly less iron than RPE/choroid samples (mean:  $603.8 \mu\text{g/g}$ ; range:  $147.7$ – $1155.0 \mu\text{g/g}$ ) ( $P < 0.0001$ ), consistent with a similar analysis in the rat retina [15]. Among the 12 donors who had both left and right eyes available for iron measurements, there was no significant intereye difference in iron levels in the retina ( $P = 0.57$ ) or in the RPE/choroid ( $P = 0.10$ ) of the same individual, although RPE/choroid samples from the same individual exhibited much greater variability than retinal samples (not shown).

Comparison of iron levels in both younger and older samples and in both sexes revealed no significant correlation between RPE/choroid iron and any of the analyzed parameters (not shown). On the other hand, age-associated and sex-associated differences in iron levels were present in the retina (Fig. 1). A significant increase was observed in retinal iron levels in older vs. younger individuals (mean (SEM):  $116$  (9.73) vs.  $76.5$  (9.15)  $\mu\text{g/g}$ ;  $P = 0.003$ ). Increasing retinal iron with age from all patients corresponded to a mean (SEM) per year increase  $0.70$  (0.22)  $\mu\text{g/g}$  ( $P = 0.001$ ).

This age-associated increase in iron was observed in retinas of both women and men. Older vs. younger females had mean (SEM) iron levels of  $137$  (13.8) vs.  $96.1$  (14.8)  $\mu\text{g/g}$  ( $P = 0.04$ ). Older vs. younger males had mean (SEM) iron levels of  $95.4$  (13.6) vs.  $57.2$  (8.06)  $\mu\text{g/g}$  ( $P = 0.016$ ).



**Fig. 1** Retinal iron levels vs. age from older and younger patients of both sexes. Female data points are presented as Xs with a hatched line representing the female regression line. Male data points are presented as diamonds with a solid line representing the male regression line.

Comparison of iron levels between women and men of all ages revealed that women had more retinal iron than men [mean (SEM): 119 (10.7) vs. 77.1 (8.14)  $\mu\text{g/g}$ ,  $P = 0.001$ ]. This sex difference existed in both younger and older age groups. In the younger group, women vs. men had mean (SEM) iron levels of 96.1 (14.8) vs. 57.2 (8.06)  $\mu\text{g/g}$  ( $P = 0.02$ ). In the older group, women vs. men had mean (SEM) iron levels of 137 (13.8) vs. 95.4 (13.6)  $\mu\text{g/g}$  ( $P = 0.03$ ). For women, the distribution of iron with age is described by the linear equation: retinal iron =  $88.1 + 0.62 \times \text{Age}$ ;  $R^2 = 0.17$ ,  $P = 0.051$ ; for men, the distribution is described as: retinal iron =  $34.8 + 0.83 \times \text{Age}$ ;  $R^2 = 0.33$ ,  $P = 0.001$  (Fig. 1).

## Discussion

Increasing susceptibility to oxidative stress is believed to be important in the development of aging and the pathogenesis of age-related diseases. Iron is essential for life, but is also a highly reactive pro-oxidant that may be an important generator of oxidative stress. Abnormal iron accumulation has been implicated in several age-related human diseases, including neurodegenerations such as age-related macular degeneration, in which excess iron has been observed in the macular retina and RPE [6,16]. Additionally, studies have shown that age is accompanied by increasing levels of serum ferritin, analyzed as a surrogate measure of body stores of iron [8,9]. Serum ferritin is an acute-phase reactant that increases in response to inflammation [10], and the age-associated increases in iron measured by serum ferritin may be confounded by the increased presence of chronic disease in the elderly. In order to conclusively determine whether iron levels within tissue increase with age, we applied atomic absorption spectrophotometry to assess quantitatively and directly iron levels in post-mortem samples of the retina, a CNS tissue, and of RPE/choroid, a tissue exposed to systemic blood flow, of various ages and from both sexes.

We found that retinal iron increases with age in both men and women, consistent with similar increases seen in serum ferritin levels. It is generally considered that women, especially before menopause, have lower levels of iron stores than age-matched men [9]. It was therefore surprising to find that women of all age groups had significantly increased retinal iron compared with men. There may be tissue-specific mechanisms of iron-handling, such that retinas, and perhaps other tissues, accumulate iron independent of serum iron levels. Mice deficient in the ferroxidases ceruloplasmin and hephaestin are anemic, but abnormal iron accumulation is observed in the eye, suggesting that there may indeed be tissue-specific mechanisms of iron regulation [16].

Additionally, there may be hormonal or sex-specific differences in iron handling. In several mouse studies, sex has been shown to affect the consequences of dietary iron manipulations, suggesting that the sex of an individual may alter the impact of different dietary intake patterns [17–19]. In a subset of human hemochromatosis patients, there exist sex-associated variations in frequency of certain human leukocyte antigen haplotypes, which correspond to differences in serum ferritin and transferrin saturation [20]. Similar disparities may exist in other genes involved in iron handling, and the possibility of sex-specific mechanisms of iron regulation warrants further investigation.

Relative to age-matched controls, retinas from patients with age-related macular degeneration have elevated iron

levels, which may contribute to the retinal degeneration. Some evidence exists that women may have a higher frequency than men of developing age-related macular degeneration and its complications [21,22]. This epidemiological difference may result, in part, from the increased retinal iron that we detected in women over men.

In all, there were three samples from the older group that had unusually high retinal iron levels (Fig. 1). Interestingly, their contralateral retinas had normal iron levels. These high iron levels may represent normal variation or may be a precursor to disease such as age-related macular degeneration.

We observed age-associated and sex-associated changes in iron in the retina but not the RPE/choroid. The retina is a CNS structure protected by a blood-brain barrier, which likely regulates iron flow into the retina. Minimal variation of measured retinal iron was found between left and right eyes from the same individual, suggesting that retinal iron is highly regulated, allowing us to observe age-related changes within a manageable sample size. On the other hand, the choroid contains a network of blood vessels whose iron levels may have been affected by subtle variations in post-mortem interval, fixation, or other aspects of sample preparation. Greater variability was found in the RPE/choroid than in the retinal iron levels in samples from the same individual (not shown), suggesting that there may indeed have been variations in sample preparation; similar studies using a larger sample size may be useful in determining whether RPE/choroid iron levels are similarly associated with age and/or sex.

## Conclusion

Iron levels within the human retina increase with age. Women have significantly more retinal iron than men at all ages.

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