Dysfunction of the Retinal Pigment Epithelium with Age: Increased Iron Decreases Phagocytosis and Lysosomal Activity

Huiyi Chen, Thomas J. Lukas, Nga Du, Genn Suyeoka, and Arthur H. Neufeld

PURPOSE. Iron accumulation with age in the retinal pigment epithelium (RPE) may be one important source of oxidative stress that contributes to age-related macular degeneration (AMD). Young and old rodent RPE/choroid were compared to assess iron homeostasis during normal aging and the effects of increased iron on the functions of retinal pigment epithelial cells.

METHODS. The iron level, mRNA expression, and protein level of iron-regulatory molecules in RPE/choroid were quantitatively compared between young and old animals. To test the effects of increased intracellular iron on the functions of retinal pigment epithelial cells, in vitro ARPE-19 cells were treated with high levels of iron and assessed for phagocytosis activity and lysosomal activity.

RESULTS. Iron level was significantly increased in the aged RPE/choroid. Ferritin and ceruloplasmin mRNAs were significantly increased in the aged RPE/choroid, whereas transferrin, transferrin receptor, and ferroportin mRNAs did not change with age. At the protein level, decreased transferrin and transferrin receptor, increased ferritin and ceruloplasmin, and unchanged ferroportin were observed in the aged RPE/choroid. Exposure of ARPE-19 cells to increased iron markedly decreased phagocytosis activity, interrupted cathepsin D processing, and reduced cathepsin D activity in retinal pigment epithelial cells.

CONCLUSIONS. The RPE/choroid of aged animals demonstrates iron accumulation and associated alterations in iron homeostasis. Iron accumulation with age may impair the phagocytosis and lysosomal functions of retinal pigment epithelial cells in the aged RPE/choroid. Therefore, age-related changes of iron homeostasis in the RPE could increase the susceptibility of the tissue to genetic mutations associated with AMD.

Iron accumulation with age in the retinal pigment epithelium (RPE) may be one important source of oxidative stress that contributes to age-related macular degeneration (AMD). Young and old rodent RPE/choroid were compared to assess iron homeostasis during normal aging and the effects of increased iron on the functions of retinal pigment epithelial cells. Methodologically, the iron level, mRNA expression, and protein level of iron-regulatory molecules in RPE/choroid were quantitatively compared between young and old animals. To test the effects of increased intracellular iron on the functions of retinal pigment epithelial cells, in vitro ARPE-19 cells were treated with high levels of iron and assessed for phagocytosis activity and lysosomal activity.

RESULTS. Iron level was significantly increased in the aged RPE/choroid. Ferritin and ceruloplasmin mRNAs were significantly increased in the aged RPE/choroid, whereas transferrin, transferrin receptor, and ferroportin mRNAs did not change with age. At the protein level, decreased transferrin and transferrin receptor, increased ferritin and ceruloplasmin, and unchanged ferroportin were observed in the aged RPE/choroid. Exposure of ARPE-19 cells to increased iron markedly decreased phagocytosis activity, interrupted cathepsin D processing, and reduced cathepsin D activity in retinal pigment epithelial cells.

CONCLUSIONS. The RPE/choroid of aged animals demonstrates iron accumulation and associated alterations in iron homeostasis. Iron accumulation with age may impair the phagocytosis and lysosomal functions of retinal pigment epithelial cells in the aged RPE/choroid. Therefore, age-related changes of iron homeostasis in the RPE could increase the susceptibility of the tissue to genetic mutations associated with AMD.

From the Forsythe Laboratory for the Investigation of the Aging Retina, Department of Ophthalmology, Northwestern University School of Medicine, Chicago, Illinois.

Supported by National Institutes of Health Grant EY12017, a generous gift from the Forsythe Foundation, and an unrestricted grant from Research to Prevent Blindness.

Submitted for publication September 10, 2008; revised November 7 and December 12, 2008; accepted February 17, 2009.

Disclosure: H. Chen, None; T.J. Lukas, None; N. Du, None; G. Suyeoka, None; A.H. Neufeld, None.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be marked ‘advertisement’ in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Corresponding author: Huiyi Chen, Forsythe Laboratory for the Investigation of the Aging Retina, Department of Ophthalmology, Northwestern University School of Medicine, Tarry 13-752, 303 E. Chicago Avenue, Chicago, IL 60611; huiyi-chen@northwestern.edu.

Investigative Ophthalmology & Visual Science. April 2009, Vol. 50, No. 4
Copyright © Association for Research in Vision and Ophthalmology

Materials and Methods

Animals

All experimental protocols were in compliance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. C57BL/6 male mice 4 and 26 months of age and Brown Norway male rats 4 and 30 months of age (National Institute on Aging, Bethesda, MD) were used in this study. We used mice for most of our work and rats for measurement of iron levels in the RPE/choroid because old mice were no longer available to us. All animals were housed under standard conditions and 12-hour cyclic light.
RPE/Choroid Dissection

Mouse and rat RPE/choroid were carefully dissected and completely separated from neural retinas and sclera. The mouse tissues were placed in RNA or protein lysis buffer. Two RPE/choroid from one mouse were pooled as one RNA sample. Four RPE/choroid from two mice were pooled as one protein sample. There were three samples from young animals and three from old animals.

Quantitative RPE/Choroid Iron and Manganese Detection

Iron levels in the RPE/choroid from three young and three old rats were determined by inductively coupled plasma-optical emission spectrophotometry (Vista-MPX ICP-OES; Varian Inc., Palo Alto, CA), as previously described.21 Manganese levels in each sample were measured at the same time as an internal control. Standards were prepared from atomic absorption standard solutions of iron and manganese (1 g/L; Thermo Fisher Scientific, Waltham, MA). Standards, blanks, and standard curves were used as previously specified.21 Serum iron of the same rat was detected (Quantichrom Iron Assay Kit; Bioassay Systems, Hayward, CA) as another internal control.

Iron Treatment of ARPE-19 Cells

Human ARPE-19 cells were purchased from American Type Culture Collection (Manassas, VA) and maintained in DMEM/F12 with 10% fetal bovine serum. Cells at complete confluence were used for experiments. Nontoxic doses of 500 μM, 1000 μM, and 2000 μM (see Fig. 4A) were added for 24 hours, and cell viability was quantitated by MTT assay (Promega, Madison, WI).

Western Blot Analysis

Mice RPE/choroid or ARPE-19 cells were lysed in the extraction buffer containing 50 mM Tris-HCl (pH 8.0), 150 mM NaCl, and 1 mM EDTA plus protease inhibitors (Pierce, Germany) and were sonicated for 5 seconds before protein concentration determination by the Bradford colorimetric assay. Fifteen micrograms of proteins were separated on SDS-PAGE and electrotransferred onto polyvinylidene difluoride membranes. The membrane was blocked in 5% nonfat milk and 0.05% Tween 20 in Tris-buffered saline (TBS) and then was incubated with primary antibodies (Table 2) diluted with primary antibodies (Table 2) at 4°C overnight. The membrane was rinsed with 0.05% Tween 20 in TBS and incubated with secondary antibody for 1 hour. Blots were developed by enhanced chemiluminescence (ECL). Relative band density was determined with ImageJ software (developed by Wayne Rasband, National Institutes of Health, Bethesda, MD; available at http://rsb.info.nih.gov/ij/index.html). β-Actin was used as loading and quality control.

Immunofluorescence Labeling

Eyes were fixed in 4% paraformaldehyde in PBS for 4 hours and embedded in paraffin. Five-micrometer-thick sections were dewaxed and rehydrated. For antigen retrieval, the sections were heated in 10 mM sodium citrate buffer (pH 6.0) at a sub-boiling temperature for 10 minutes, followed by cooling for 30 minutes. ARPE-19 cells were fixed in 4% paraformaldehyde in PBS for 10 minutes. Tissue sections or coverslips were incubated with primary antibodies (Table 2) diluted in 5% BSA in PBS overnight at 4°C. Sections incubated with 5% BSA in PBS without primary antibody were used as negative controls. After several washes, samples were incubated with the secondary antibody. After washing with PBS, slides were mounted with medium containing DAPI (Vectashield; Vector Laboratories, Burlingame, CA). Staining was performed by inductively coupled plasma-optical emission spectrophotometry (Vista-MPX ICP-OES; Varian Inc., Palo Alto, CA), as previously described.21

Table 1. Gene-Specific Primers Used in the Study

<table>
<thead>
<tr>
<th>Gene</th>
<th>Forward Primer</th>
<th>Reverse Primer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tf</td>
<td>ggagatgcaaccaaatggttttc</td>
<td>agcaacctctcatgtta</td>
</tr>
<tr>
<td>TrfR</td>
<td>ctggaaacctgtgatccatca</td>
<td>ggagttggtggcacagatat</td>
</tr>
<tr>
<td>L-Ft</td>
<td>cgcgctccttacaagtttc</td>
<td>ttaggcttaacccgcggagag</td>
</tr>
<tr>
<td>H-Ft</td>
<td>ttagaagagctggaaaccagcg</td>
<td>gacaacccaccttgatccatcg</td>
</tr>
<tr>
<td>Fpn</td>
<td>cttgctacgctgcggcttttg</td>
<td>ggaagctgcacaccatgataag</td>
</tr>
<tr>
<td>Cp</td>
<td>ggcccagacgtctcaccataaan</td>
<td>catggactatggctgctcaca</td>
</tr>
<tr>
<td>I85</td>
<td>gtaacccgttgaaacccatt</td>
<td>ccaaaccaacctcggagac</td>
</tr>
</tbody>
</table>

Table 2. Primary Antibodies Used in the Study

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Source</th>
<th>Type of Antibody</th>
<th>Dilution (WB)</th>
<th>Dilution (IF)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tf</td>
<td>Santa Cruz</td>
<td>Rabbit polyclonal</td>
<td>1:800</td>
<td>1:200</td>
</tr>
<tr>
<td>TrfR</td>
<td>Zymed</td>
<td>Mouse monoclonal</td>
<td>1:10,000</td>
<td>1:200</td>
</tr>
<tr>
<td>Ft</td>
<td>Sigma</td>
<td>Rabbit polyclonal</td>
<td>1:2,000</td>
<td>1:800</td>
</tr>
<tr>
<td>Fpn</td>
<td>Orbigen</td>
<td>Rabbit polyclonal</td>
<td>1:500</td>
<td>1:400</td>
</tr>
<tr>
<td>Cp</td>
<td>BD Biosciences</td>
<td>Mouse monoclonal</td>
<td>1:500</td>
<td>1:200</td>
</tr>
<tr>
<td>Cat D</td>
<td>GeneTex</td>
<td>Rabbit polyclonal</td>
<td>1:10,000</td>
<td>—</td>
</tr>
<tr>
<td>ZO-1</td>
<td>Zymed</td>
<td>Rabbit polyclonal</td>
<td>—</td>
<td>1:200</td>
</tr>
<tr>
<td>β-actin</td>
<td>Sigma</td>
<td>Mouse monoclonal</td>
<td>1:20,000</td>
<td>—</td>
</tr>
</tbody>
</table>

WB, Western blot; IF, immunofluorescence labeling; Tf, transferrin; TrfR, transferrin receptor; Ft, ferritin; Fpn, ferroportin; Cp, ceruloplasmin; Cat D, cathepsin D.
formed on four mice in each age group and was repeated three times for each antibody and each age. The results were consistent.

**Lysosomal Enzyme Activities In Vitro**

Cathepsin D activity was measured in retinal pigment epithelial cell extracts with the use of a kit containing a fluorogenic peptide substrate peptide, MOCAc-Gly-Lys-Pro-Ile-Leu-Phe-Phe-Arg-Leu-Lys (DNP)-D-Arg-NH2 (Sigma), reaction buffer (pH 4.0), and standards. Reactions were initiated by the addition of substrate, and the kinetics of substrate hydrolysis was measured at 340/460 nm using a fluorescent plate reader at 37°C for 15 minutes with data points collected every 120 seconds. Each assay was repeated three times. Data were imported for analysis (Prism; GraphPad Software Inc., San Diego, CA) and determination of initial rates, and normalization to total protein was assayed.

**Live Cell Lysosomal Enzyme Assay**

The targeted peptide substrate (R9-Cat D) was developed by Fischer et al. After iron treatment, cells were washed and incubated with serum- and dye-free culture media containing 2 μM R9-Cat D peptide for 1 hour. Media were removed, and cells washed twice with PBS. Then fluorescein (485/525) and rhodamine fluorescence (580/620) was sequentially recorded using a microplate reader (Tecan). Each assay was repeated three times.

**RESULTS**

**Increased Iron Levels in Aged RPE/Choroid**

ICP-OES results showed that the iron levels were significantly increased by threefold in the RPE/choroid of old rats (195.4 ± 35 μg/g [dry weight]) compared with the RPE/choroid of young rats (65.0 ± 8 μg/g; Fig. 1A). As an internal control, we also measured manganese levels in the same samples. Manganese levels in the young and aged RPE/choroid were 2.1 ± 0.04 μg/g and 2.0 ± 0.03 μg/g, respectively, and were not significantly different (Fig. 1B). To determine systemic iron levels, we measured the serum iron. There was no significant difference between young and old rats (181.6 ± 25.3 μg/dL and 164.4 ± 51.7 μg/dL, respectively; Fig. 1C). Thus, there was a significant increase in the tissue levels of iron in the RPE/choroid of old rats.

**Changes in Iron-Regulatory Molecules in Aged RPE/Choroid**

To maintain iron homeostasis locally in tissues, a series of iron-regulatory proteins tightly control cellular iron uptake, storage, intracellular distribution, and export. The main iron-regulatory molecules include transferrin (Tf), transferrin receptor (TrfR), ferritin (Ft), ferroportin (Fpn), and ceruloplasmin (Cp). Tf is the major iron transport protein. TrfR is the cell surface receptor for Tf, and the binding of Tf to TrfR enables the cells to take up iron by endocytosis. Intracellular iron is stored mainly in Ft, which is composed of 24 subunits of ferritin light chain (L-Ft) and ferritin heavy chain (H-Ft). Fpn is the only putative iron exporter identified to date, and Cp functions as a ferroxidase participating in the release of iron from cells. We compared the gene expressions, protein levels, and cellular locations of these iron-regulatory molecules in the RPE/choroid of young and old mice by real time RT-PCR, Western blot, and immunofluorescence staining.

Real-time RT-PCR showed the gene expression levels of L-Ft, H-Ft, and Cp were significantly increased in the aged RPE/choroid (P < 0.05; Fig. 2), but Tf, TrfR, and Fpn mRNA expression did not show significant differences between young and old mice (P > 0.05; Fig. 2).

Although Tf and TrfR mRNA were not changed with age, the amount of protein synthesized from both Tf and TrfR were significantly decreased in the aged RPE/choroid, suggesting posttranscriptional downregulation of Tf and TrfR protein synthesis in the old mice (P < 0.05; Figs. 3A, 3B). The protein levels of Ft and Cp were significantly increased in the aged RPE/choroid (P < 0.05; Figs. 3A, 3B) and were consistent with the changes in mRNA level. There was no significant change in Fpn protein level in aged RPE/choroid (P > 0.05; Figs. 3A, 3B). Immunofluorescence labeling showed all these iron-regulatory proteins were present in retinal pigment epithelial cells in young and old mice. Figures 3E and 3F show the labeling for Ft in retinal pigment epithelial cells in young and old mice. In the young eye, slight labeling for Ft is present in the RPE. In the old eye, dense particulate labeling for Ft can be seen throughout the RPE layer and in endothelial cells of the choroid, near the
Bruch membrane. Other molecules showed similar cellular localizations (data not shown).

Decrease in the Phagocytosis Activity of RPE Cells Due to Elevated Intracellular Iron

Our results showed increased iron in the RPE/choroid of aged rats compared with that of young rats. This age-related, increased intracellular iron may cause dysfunction of retinal pigment epithelial cells. With the use of a human retinal pigment epithelial cell line, ARPE-19, we sought to determine whether increased intracellular iron would alter the physiological functions of RPE.

First, we determined in vitro the sublethal concentrations of Fe-NTA that would not cause the death of ARPE-19 cells. NTA is an iron carrier, and Fe-NTA enters cells rapidly and independently of TrfR-mediated iron uptake. Increased Fe-NTA concentrations in the culture media increase the intracellular iron level.28 We found that increasing Fe-NTA levels up to 2000 \( \mu M \) in the media did not cause a significant decrease in cell viability (\( P > 0.05 \); Fig. 4A). Only 3000 \( \mu M \) Fe-NTA had a small cytotoxic effect on the ARPE-19 cells (\( P < 0.05 \)). Therefore, we chose the concentrations of 500 \( \mu M \), 1000 \( \mu M \), and 2000 \( \mu M \) Fe-NTA (Fig. 4A, arrows) for further experiments.

We detected changes in the two major iron-regulatory proteins, TrfR and Ft, under increased iron conditions in vitro. Western blot showed the increased iron in vitro downregulated TrfR and upregulated Ft proteins (Fig. 4B), consistent with the physiological response of retinal pigment epithelial cells to increased intracellular iron and with the age-related changes in the presence of excess iron in the aged RPE/choroid (Fig. 3A). We suggest that this model of iron loading mimics, at least in part, the intracellular iron accumulation and altered iron homeostasis that occurs with age.

Phagocytosis of POS is one of the most important functions of retinal pigment epithelial cells. Impaired phagocytosis function of RPE has been associated with the pathogenesis of AMD. To test the effect of increased iron on the phagocytic activity of retinal pigment epithelial cells, we performed phagocytosis assays that quantified the uptake of fluorescein-labeled POS (FITC-POS) by ARPE-19. Exposure to FITC-POS demonstrated continuous increased uptake of POS by ARPE-19 for 6 hours under control conditions. However, preincubating the cells in media containing a nontoxic concentration of 1000 \( \mu M \) Fe-NTA drastically lowered the rate of uptake of FITC-POS at all time points (Fig. 4C). We found that the effects of elevated iron on the phagocytosis activity of retinal pigment epithelial cells were concentration dependent. With pretreatment of 500 \( \mu M \), 1000 \( \mu M \), and 2000 \( \mu M \) Fe-NTA to the ARPE-19 cells, POS uptake was decreased by approximately 39%, 45%, and 78% of the total uptake, respectively, at 3 hours (Fig. 4D). Immunofluorescence labeling further confirmed that there was less POS uptake in the iron-treated cells than in the nontreated cells (Figs. 4E, 4F).
Decrease in the Lysosomal Activity of RPE Cells Due to Elevated Intracellular Iron

The key lysosomal enzyme involved in the lysosomal processing of POS is cathepsin D (Cat D). Retinal pigment epithelial cells were treated with Fe-NTA for 24 hours and fed with POS for 3 hours, and then Cat D protein was measured. Western blot analysis showed there were two bands of Cat D. One band was the active form of mature Cat D at 34 kDa, and the other band was the inactive form of pro-Cat D at 48 to 52 kDa.

Increased iron with or without exposure to POS caused a significant increase of pro-Cat D (Fig. 5A), quantified by densitometry (Fig. 5B).

Cat D is a protease that requires the acidic environment of the lysosome for its activity. Although the amount of mature Cat D protein was not visibly affected on the Western blot by iron, the activity of Cat D in the lysates from retinal pigment epithelial cells treated with 500 to 2000 μM Fe-NTA with or without POS was significantly decreased (Fig. 5C). POS alone did not affect Cat D activity in the lysate. To determine whether the decrease in Cat D activity measured in cell lysates could also be observed in live cells, we incubated the cells with a Cat D substrate peptide, R9-Cat D. The peptide has a fluorescein at the C terminus and a tetramethylrhodamine at the N terminus of the Cat D cleavage sequence. The peptide exhibits different fluorescence, depending on its cleavage status. The red rhodamine fluorescence represents the amount of R9-Cat D inside the cells, and the green fluorescein fluorescence represents the amount of the C-terminal cleaved fragments. Therefore, the normalized ratio of green to red fluorescence represents the Cat D activity to cleave R9-Cat D in the lysosome compartments of the live cells. Our result showed that iron-treated cells with or without POS exhibited significantly decreased Cat D activity in live cells (Fig. 5D).

When R9-Cat D was used as a substrate in live cells, adding POS decreased the amount of fluorescence, most likely because of competition of R9-Cat D and protein from the POS for the enzyme.

**DISCUSSION**

Accumulation of redox-active iron in the retina is associated with several retinal degenerative diseases, including AMD, for which age is a major risk factor. Thus, the changes of iron homeostasis with age may contribute to the underlying molecular basis of increased risk associated with age. RPE dysfunction is particularly relevant to the pathogenesis of AMD. Here, we determined the changes of iron levels and the expression of iron-regulatory molecules in RPE/choroid that occur with nor-
mal aging and assessed the effects of iron on several of the key physiological functions of retinal pigment epithelial cells in vitro. Our results suggest there are age-related changes of iron homeostasis in the normal, aged RPE/choroid, including accumulation of iron and associated changes of iron-regulatory molecules. Furthermore, at least in vitro and likely in vivo, elevated levels of intracellular free iron decrease the phagocytic and Cat D activities of retinal pigment epithelial cells.

Iron Accumulation in the RPE/Choroid with Age

Because iron is absorbed from the diet and little is excreted, iron accumulates and total body iron level increases with age. For example, iron accumulates in many regions of the brain with age, and local mismanagement of iron homeostasis is associated with age-related neurodegeneration, such as Parkinson disease and Alzheimer disease. Age-related increases in iron level have been observed in the neural retinas of humans and rodents, and previous work on human RPE/choroid demonstrated great variability of samples, and no clear conclusion was made. Our study indeed showed an age-related accumulation of iron in RPE/choroid of rat that was independent of serum iron levels. These results were consistent with many other studies showing the lack of correlation between iron levels in local tissues and serum iron levels. Mice deficient in Cp and hephaestin are anemic, but abnormal iron accumulation is found in their retinas. Patients with aceruloplasminemia are anemic but have retinal iron overload. In our study, although the systemic iron level was within the normal range in healthy aged animals, iron accumulation was increased in the aged RPE/choroid, suggesting a tissue-specific, iron-regulating mechanism.

We previously reported a modest but significant increase in iron level in the aged rat neural retina compared with the young rat neural retina. Surprisingly, the difference in iron level in the RPE/choroid between young and old rats (3.0-fold) was higher than that in the neural retina (1.3-fold). Iron is taken up at the basolateral surface of retinal pigment epithelial cells and is transported to the apical surfaces of retinal pigment epithelial cells, where it is released to the neural retina. Therefore, iron flow to the neural retina may be limited and controlled by the RPE layer. The RPE, which maintains the outer blood-retinal barrier, may protect the neural retina from the effects of systemic iron overload or deficiency.

Iron level in the local tissue is tightly regulated by a group of iron-regulatory molecules. Iron is taken up by retinal pigment epithelial cells through Tf-mediated uptake after binding to TrfR. Intracellular iron is sequestered by Ft and stored as a nontoxic form. The iron that is not used or stored by the cell is exported by Fpn with the assistance of Cp. In our study, although the systemic iron level was within the normal range in healthy aged animals, iron accumulation was increased in the aged RPE/choroid, suggesting a tissue-specific, iron-regulating mechanism.

We previously reported a modest but significant increase in iron level in the aged rat neural retina compared with the young rat neural retina. Surprisingly, the difference in iron level in the RPE/choroid between young and old rats (3.0-fold) was higher than that in the neural retina (1.3-fold). Iron is taken up at the basolateral surface of retinal pigment epithelial cells and is transported to the apical surfaces of retinal pigment epithelial cells, where it is released to the neural retina. Therefore, iron flow to the neural retina may be limited and controlled by the RPE layer. The RPE, which maintains the outer blood-retinal barrier, may protect the neural retina from the effects of systemic iron overload or deficiency.

Iron level in the local tissue is tightly regulated by a group of iron-regulatory molecules. Iron is taken up by retinal pigment epithelial cells through Tf-mediated uptake after binding to TrfR. Intracellular iron is sequestered by Ft and stored as a nontoxic form. The iron that is not used or stored by the cell is exported by Fpn with the assistance of Cp. In our study, although the systemic iron level was within the normal range in healthy aged animals, iron accumulation was increased in the aged RPE/choroid, suggesting a tissue-specific, iron-regulating mechanism.

We previously reported a modest but significant increase in iron level in the aged rat neural retina compared with the young rat neural retina. Surprisingly, the difference in iron level in the RPE/choroid between young and old rats (3.0-fold) was higher than that in the neural retina (1.3-fold). Iron is taken up at the basolateral surface of retinal pigment epithelial cells and is transported to the apical surfaces of retinal pigment epithelial cells, where it is released to the neural retina. Therefore, iron flow to the neural retina may be limited and controlled by the RPE layer. The RPE, which maintains the outer blood-retinal barrier, may protect the neural retina from the effects of systemic iron overload or deficiency.

Iron level in the local tissue is tightly regulated by a group of iron-regulatory molecules. Iron is taken up by retinal pigment epithelial cells through Tf-mediated uptake after binding to TrfR. Intracellular iron is sequestered by Ft and stored as a nontoxic form. The iron that is not used or stored by the cell is exported by Fpn with the assistance of Cp. In our study, although the systemic iron level was within the normal range in healthy aged animals, iron accumulation was increased in the aged RPE/choroid, suggesting a tissue-specific, iron-regulating mechanism.

We previously reported a modest but significant increase in iron level in the aged rat neural retina compared with the young rat neural retina. Surprisingly, the difference in iron level in the RPE/choroid between young and old rats (3.0-fold) was higher than that in the neural retina (1.3-fold). Iron is taken up at the basolateral surface of retinal pigment epithelial cells and is transported to the apical surfaces of retinal pigment epithelial cells, where it is released to the neural retina. Therefore, iron flow to the neural retina may be limited and controlled by the RPE layer. The RPE, which maintains the outer blood-retinal barrier, may protect the neural retina from the effects of systemic iron overload or deficiency.

Iron level in the local tissue is tightly regulated by a group of iron-regulatory molecules. Iron is taken up by retinal pigment epithelial cells through Tf-mediated uptake after binding to TrfR. Intracellular iron is sequestered by Ft and stored as a nontoxic form. The iron that is not used or stored by the cell is exported by Fpn with the assistance of Cp. In our study, although the systemic iron level was within the normal range in healthy aged animals, iron accumulation was increased in the aged RPE/choroid, suggesting a tissue-specific, iron-regulating mechanism.

We previously reported a modest but significant increase in iron level in the aged rat neural retina compared with the young rat neural retina. Surprisingly, the difference in iron level in the RPE/choroid between young and old rats (3.0-fold) was higher than that in the neural retina (1.3-fold). Iron is taken up at the basolateral surface of retinal pigment epithelial cells and is transported to the apical surfaces of retinal pigment epithelial cells, where it is released to the neural retina. Therefore, iron flow to the neural retina may be limited and controlled by the RPE layer. The RPE, which maintains the outer blood-retinal barrier, may protect the neural retina from the effects of systemic iron overload or deficiency.

Iron level in the local tissue is tightly regulated by a group of iron-regulatory molecules. Iron is taken up by retinal pigment epithelial cells through Tf-mediated uptake after binding to TrfR. Intracellular iron is sequestered by Ft and stored as a nontoxic form. The iron that is not used or stored by the cell is exported by Fpn with the assistance of Cp. In our study, although the systemic iron level was within the normal range in healthy aged animals, iron accumulation was increased in the aged RPE/choroid, suggesting a tissue-specific, iron-regulating mechanism.

We previously reported a modest but significant increase in iron level in the aged rat neural retina compared with the young rat neural retina. Surprisingly, the difference in iron level in the RPE/choroid between young and old rats (3.0-fold) was higher than that in the neural retina (1.3-fold). Iron is taken up at the basolateral surface of retinal pigment epithelial cells and is transported to the apical surfaces of retinal pigment epithelial cells, where it is released to the neural retina. Therefore, iron flow to the neural retina may be limited and controlled by the RPE layer. The RPE, which maintains the outer blood-retinal barrier, may protect the neural retina from the effects of systemic iron overload or deficiency.
for iron storage, and convert toxic ferrous iron to nontoxic ferric iron by increasing Cp. Changes in the iron-regulatory proteins TrfR and Ft are known to be regulated by a well-characterized regulatory system—iron-responsive elements (IREs)/iron-regulatory proteins (IRPs)—that senses intracellular iron levels. In iron deficiency, binding of IRPs to the IRE inhibits the translation of Ft and protects TrfR mRNA from rapid degradation, thus leading to decreased Ft and increased TrfR. On the contrary, in iron overload, IRPs are inactivated or degraded, which results in increased translation of Ft and decreased translation of TrfR. Our results, which showed increased synthesis of Ft and decreased synthesis of TrfR, suggest that the iron-regulatory molecules were posttranscriptionally regulated in the aged RPE/choroid under conditions of iron overload.

Our findings demonstrate that, in the RPE/choroid of rodents, as a normal, functional, age-related change, there is an accumulation of iron and an associated phenotypic alteration in iron homeostasis. Although these changes in iron homeostasis may not cause the death of retinal pigment epithelial cells, they may cause impairment of important physiological functions of retinal pigment epithelial cells.

**Decrease of Physiological Activities of RPE Cells Due to Increased Intracellular Iron**

Important functions of the RPE are phagocytosis of the shed tips of the POS and breakdown of the damaged photoreceptor proteins and lipids. However, the age-related accumulation of iron in RPE may impair these physiological functions. We used an in vitro retinal pigment epithelial cell line, ARPE-19, to determine the effects of sublethal iron accumulation on cellular functions. ARPE-19 is a human retinal pigment epithelial cell line that has been widely used to model retinal pigment epithelial cells for in vitro experiments. We increased intracellular free iron by using Fe-NTA in the media.

Changes in the iron-regulatory proteins TrfR and Ft are associated with decreased functions and degenerative changes. Defects in POS phagocytosis appear to be age-related, binding of IRPs to the IRE inhibits the translation of Ft and protects TrfR mRNA from rapid degradation, thus leading to decreased Ft and increased TrfR. On the contrary, in iron overload, IRPs are inactivated or degraded, which results in increased translation of Ft and decreased translation of TrfR. Our results, which showed increased synthesis of Ft and decreased synthesis of TrfR, suggest that the iron-regulatory molecules were posttranscriptionally regulated in the aged RPE/choroid under conditions of iron overload.

**References**


49. Lockwood TD. Responsiveness of parasite Cys His proteases to iron redox. Parasitol Res. 2006;100:175–181.