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OMICS Group International is a pioneer and leading science event organizer, which publishes around 400 open access journals and conducts over 300 Medical, Clinical, Engineering, Life Sciences, Phrama scientific conferences all over the globe annually with the support of more than 1000 scientific associations and 30,000 editorial board members and 3.5 million followers to its credit.

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National Eye Institute

Multiple A2E treatment leads to melanization of OS-challenged ARPE-19 cells: A model of normally aging RPE

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Lipofuscin accumulates normally in the RPE with age

- Lipofuscin is an autofluorescent, membrane-bound intracellular material
- With advancing age, there is a marked increase in the lipofuscin granule content of human retinal pigment epithelial (RPE) cells

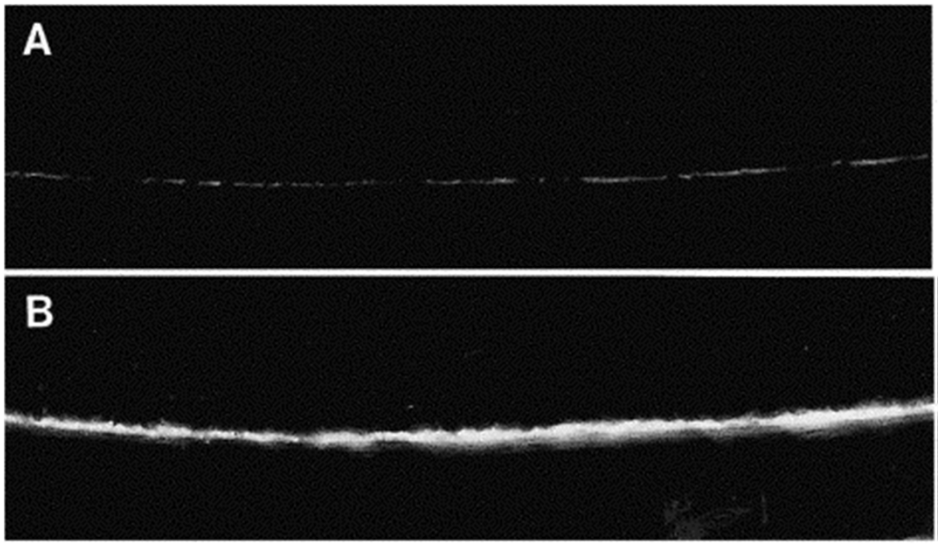
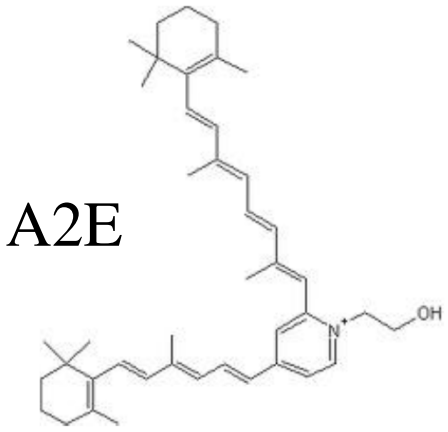


Fig. 3 Fluorescence micrographs showing the age-related accumulation of lipofuscin in the RPE. Micrographs are of cryostat sections of retinas from: (A) 26-week-old; and (B) 117-week-old albino rats.

Martin L Katz , Archives of Gerontology and Geriatrics, Volume 34, Issue 3, 2002, 359 - 370
http://dx.doi.org/10.1016/S0167-4943(02)00012-2

Increased accumulation of A2E in the RPE during the development of Macular Dystrophies.

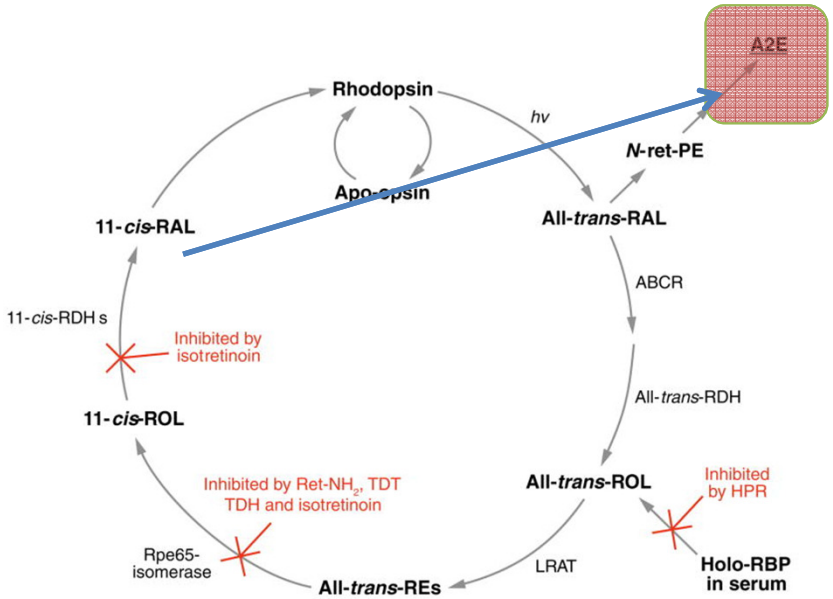
- Lipofuscin accumulation is implicated in pathology of several retinal degeneration diseases such as Vitelliform Macular Dystrophy (VMD), Stargardt macular dystrophy, Stargardt-like macular dystrophy (STGD3) and Age-Related Macular Degeneration (AMD).
- Accelerated accumulation of lipofuscin in RPE precedes visual loss in the Stargardt's patients.
- A2E and its oxidation products are major components of lipofuscin in the RPE.



A2E

A2E is produced from two molecules of vitamin A aldehyde (retinal) and one molecule of ethanolamine.

A2E, a byproduct of the visual cycle, accumulates in RPE lysosomes



A2E is a byproduct of the visual cycle.

It was widely accepted that A2E formed from all-*trans* retinal but recently 11-*cis* retinal was proposed to be the source. (N.P. Boyer et al. 2012).

Majority of the A2E fed to the RPE accumulated in the lysosomes (Holz et al. 1999, Lakkaraju et al. 2007, Vives-Bauza et al. 2008)

Striking drop in latency of the lysosome is observed at concentrations above 2 µM A2E (Shutt et al. 2002). (A2E leakage)

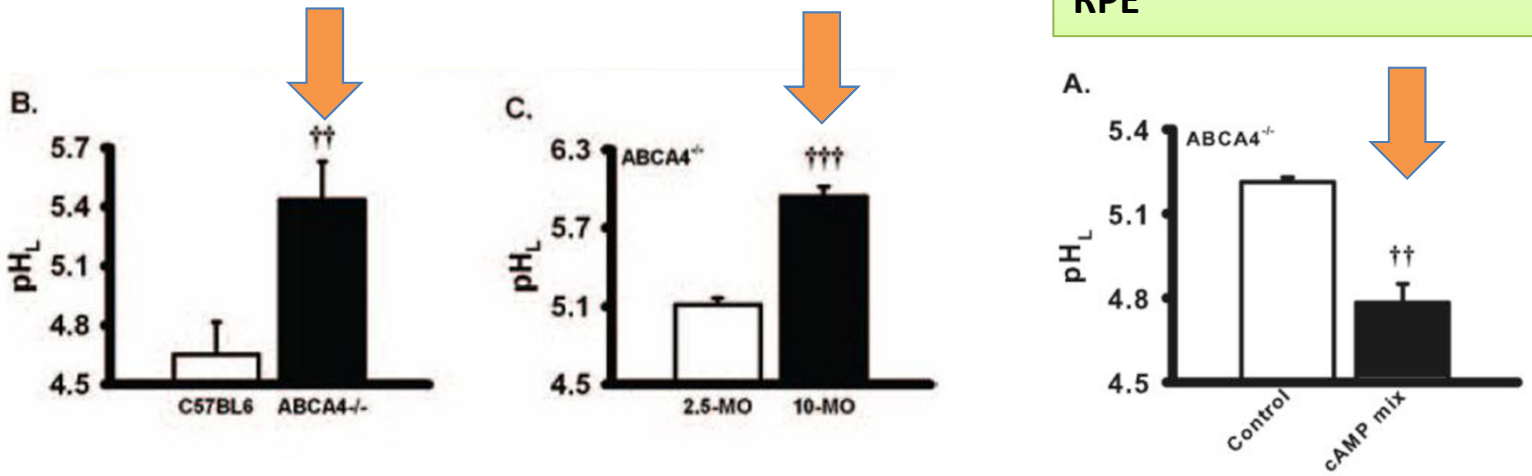
pH is increased in lysosomes from RPE of ABCA4-/- mice

Normal lysosomal pH is 4.5

RPE from ABCA4-/- mouse pH is increased (5.5)

Aged RPE ABCA4-/- pH is further increased (5.9)

cAMP-activating mix decreased pH to normal levels (4.7) in ABCA4-/- RPE



Restoration of Lysosomal pH in RPE Cells from Cultured Human and ABCA4$^{-/-}$ Mice: Pharmacologic Approaches and Functional Recovery

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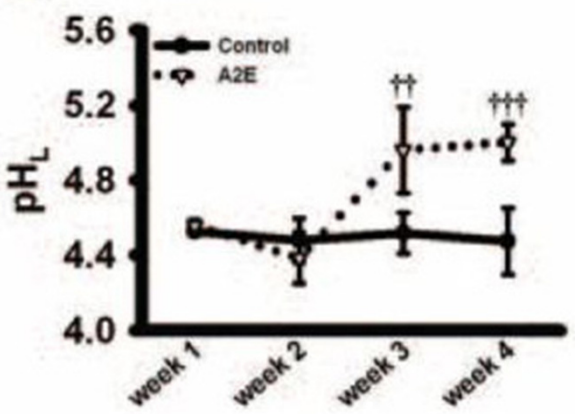
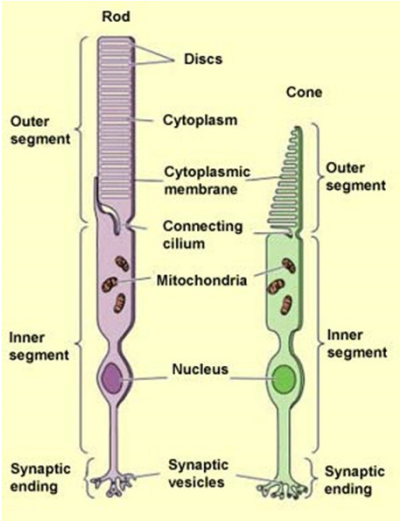
Restoring normal pH leads to the improvement of OS clearance in ARPE-19 /A2E model





Three weeks treatment of ARPE19 cells (1-2 weeks after seeding) with low doses of A2E (14 nM twice/week for 3 weeks) cause increase in lysosomal pH from 4.7 to 5.5

Treatment with cell permeable analogues of cAMP (chlorophenylthio-cAMP or cpt-cAMP) and 8-bromo-cAMP restored pH to normal levels in compromised cells

This led to improved clearance of outer segments (OS) in ARPE-19 cells

Effects of A2E accumulation in ARPE-19 cells

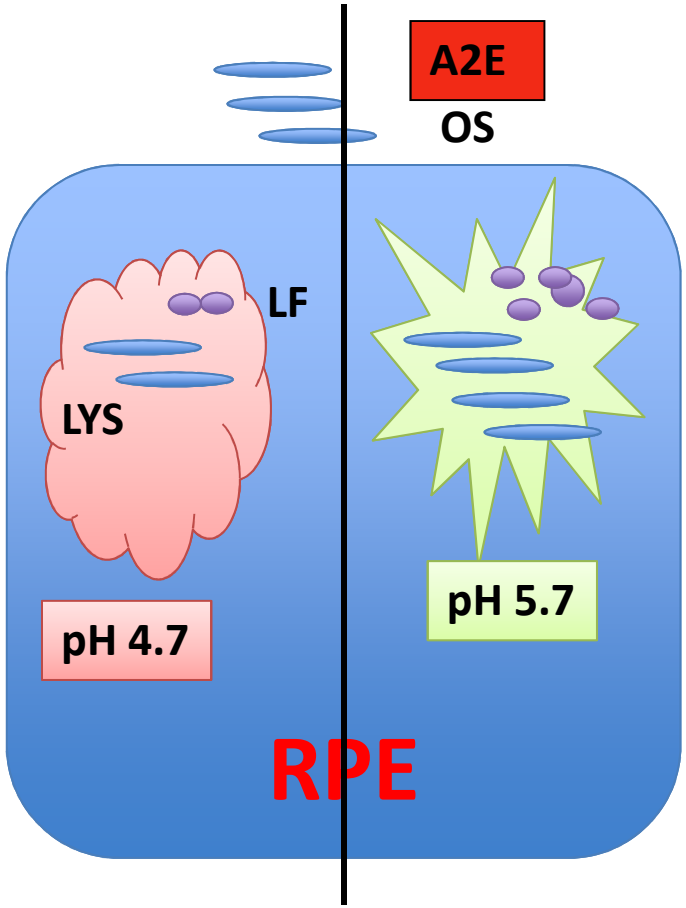
Conflicting data in the literature:

Low concentrations of A2E affects lysosomal function, increase lysosomal pH, inhibit the ATP-driven proton pump and decrease activity of the proteolytic enzymes, which impairs degradation of OS
(Holz et al., 1999 (A2E/LDL for 4 weeks <1μM) Bergmann et al., 2004 (4 weeks after confluency, less than 2 μM A2E/LDL))

Some of the studies reported no connection between A2E accumulation and pH changes in lysosomes of RPE
(Vives-Bauza C,et al.J Biol Chem. 2008, (6-7 days after seeding at 50% of confluency, 10μM A2E)
Lakkaraju A, Finnemann SC, Rodriguez-Boulan E. PNAS. 2007, (6 weeks after confluency, a single 15-μM dose or three 5-μM doses over 7 days).

What could be the explanation?

Development of an aging RPE model using long term feeding with low doses of A2E



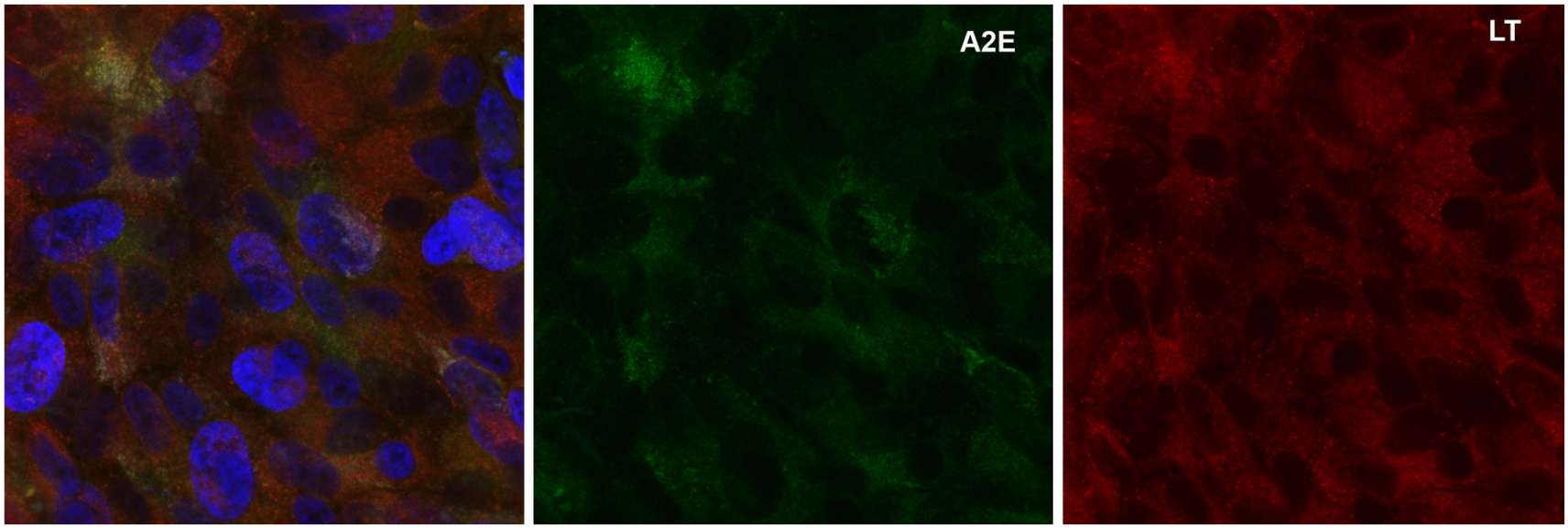
Important outcomes :

- ***Accumulation of A2E in lysosomes of treated RPE***
- ***Increasing cell stress without cell death***
- ***Modulation of lysosomal pH (measured by Lysosensors)***
- ***Efficiency of the lysosomal enzymes (measured by Sensolyte 390, PNPP).***
- ***OS processing by RPE***
- ***Degeneration or compensatory effects***

Key points for the physiological RPE model

- ARPE-19 cells were used 3 weeks after reaching confluence (**differentiated** post-mitotic cells with tight junctions as RPE).
- We used a scheme of **multiple** feedings of A2E.
- A2E was added under yellow light conditions (~ 60 lux) and left overnight on the cells.
- Concentration of A2E used for the RPE in repeated feeding: 100 nM, 1µM, 10µM. Localization in the cells, visualization, toxicity after repeated feeding in the presence of different concentrations of oxidative stimuli (HQ 0-500 µM).
- N**on-proapoptotic** concentration of A2E was used

A2E and Lysotracker (LT) are partially colocalized in ARPE-19 postconfluent cultures fed once with 10 µM A2E



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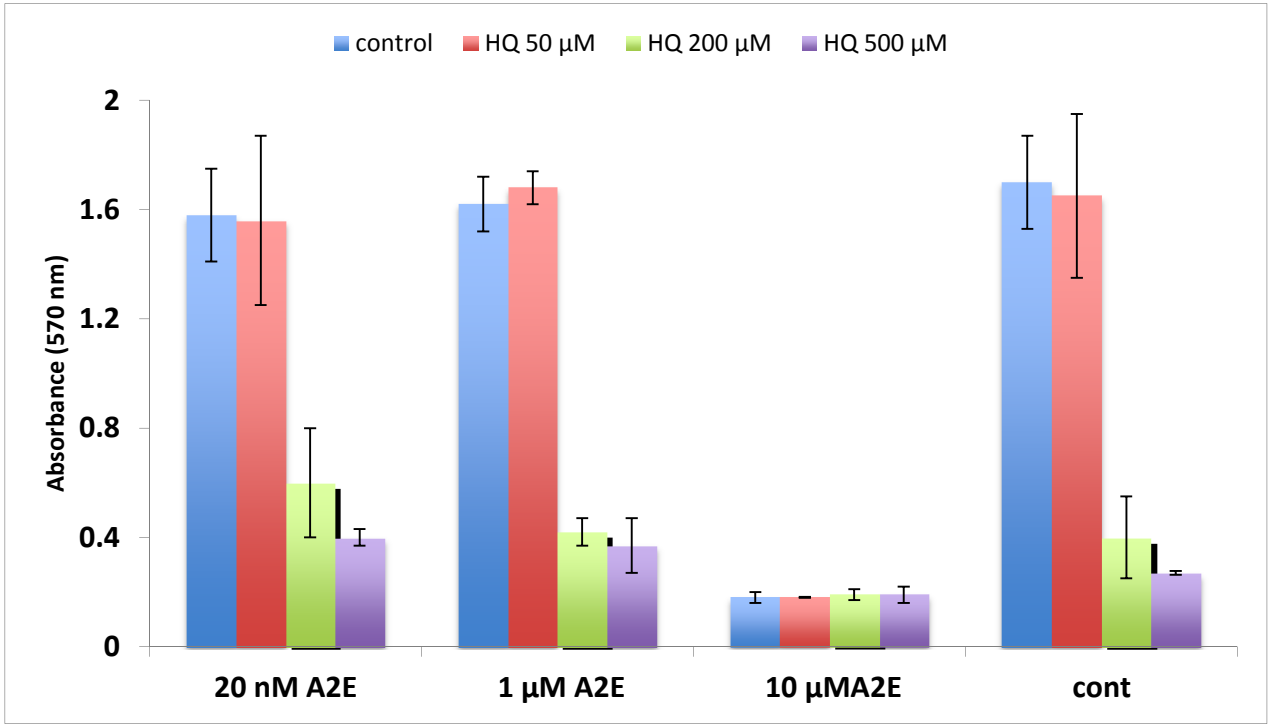
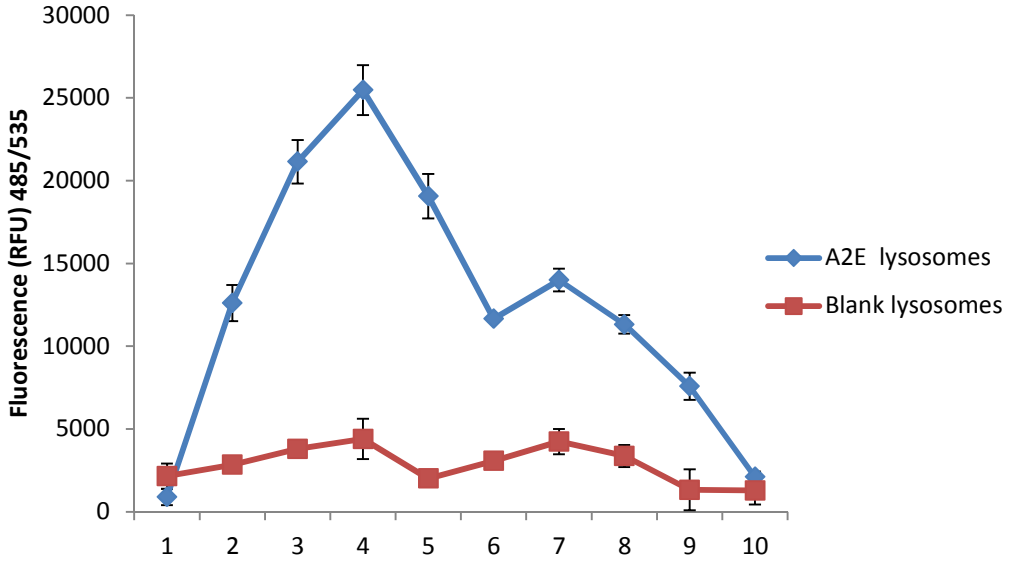
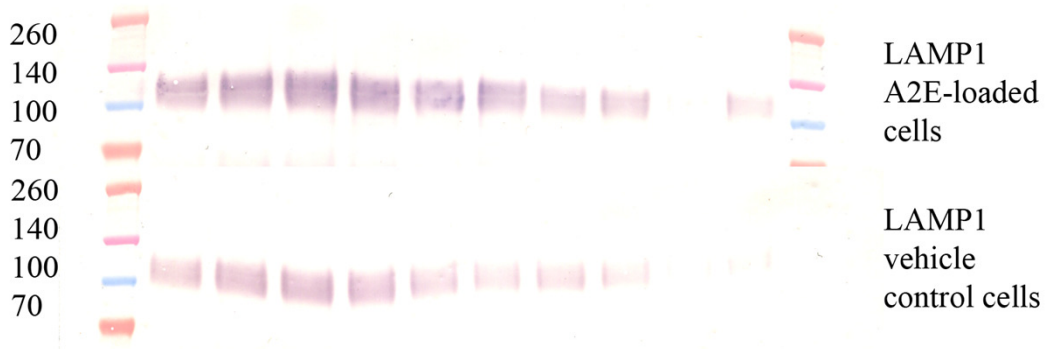
Viability of ARPE-19 after feeding with A2E

- High concentration of A2E 10µM cause cell death after several feedings.
- Viability of cells fed with multiple doses of 20 nM and 1 µM of A2E is similar in response to different concentrations oxidative stimuli (hydroquinone 50-500 µM for 16 hours and 24 hours recovery).
- Multiple A2E treatment at 1 µM is non-proapoptotic



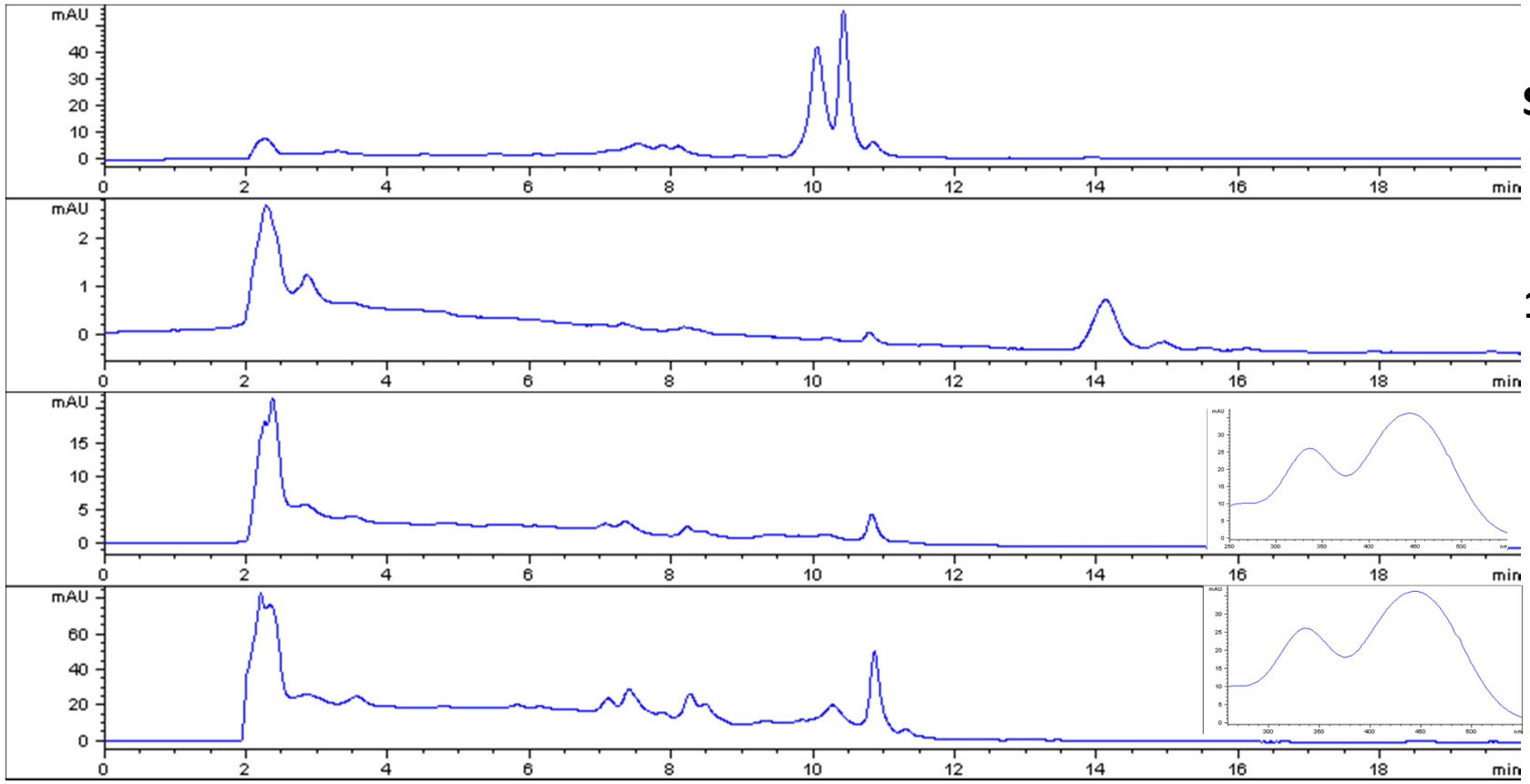
A2E is stored in lysosomes in multiple feeding experiments.



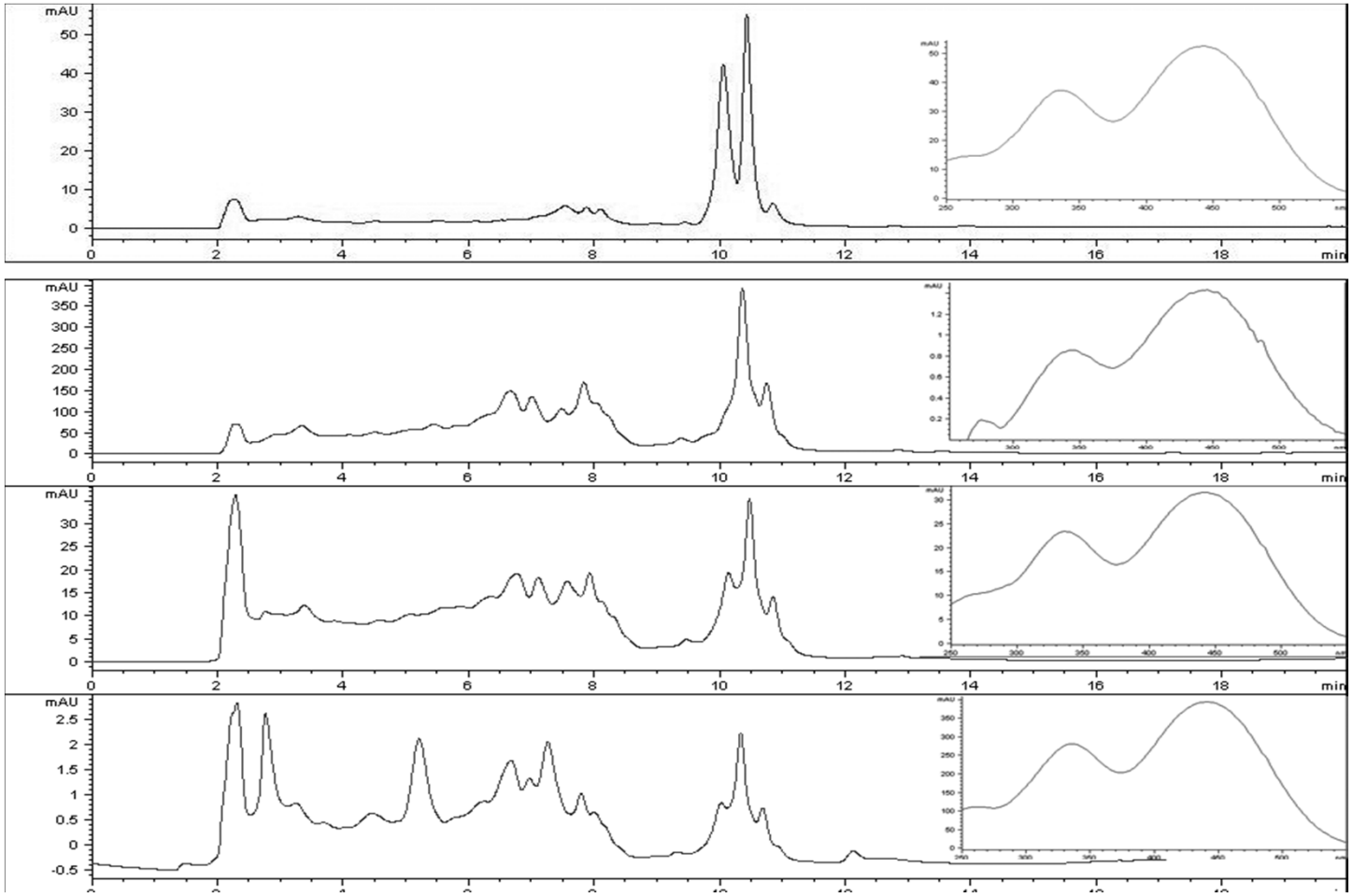


- ARPE19 cells were fed multiple times (5) during 3 weeks with 1 µM A2E in DMSO or DMSO vehicle
- Lysosomal fractions from these cells were isolated.
- A2E fluorescence (485/535) was assessed

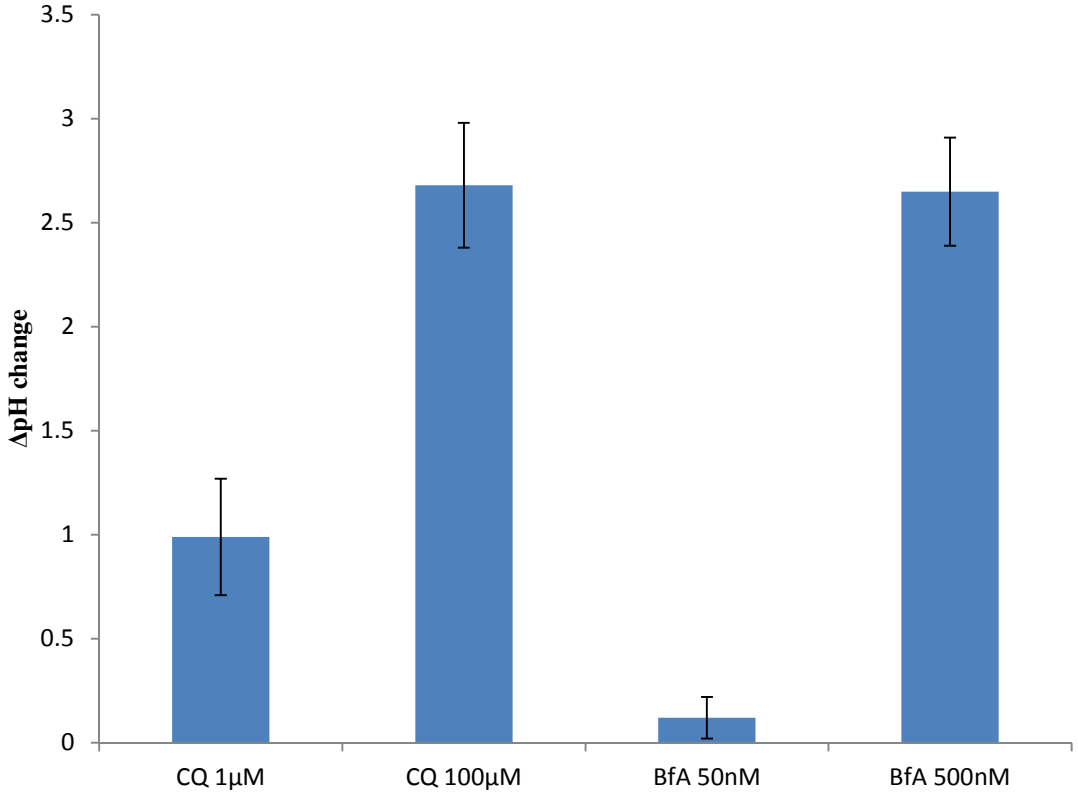
HPLC detection of A2E in single feed ARPE-19 cells



HPLC detection of A2E in multiple feed ARPE-19 cells

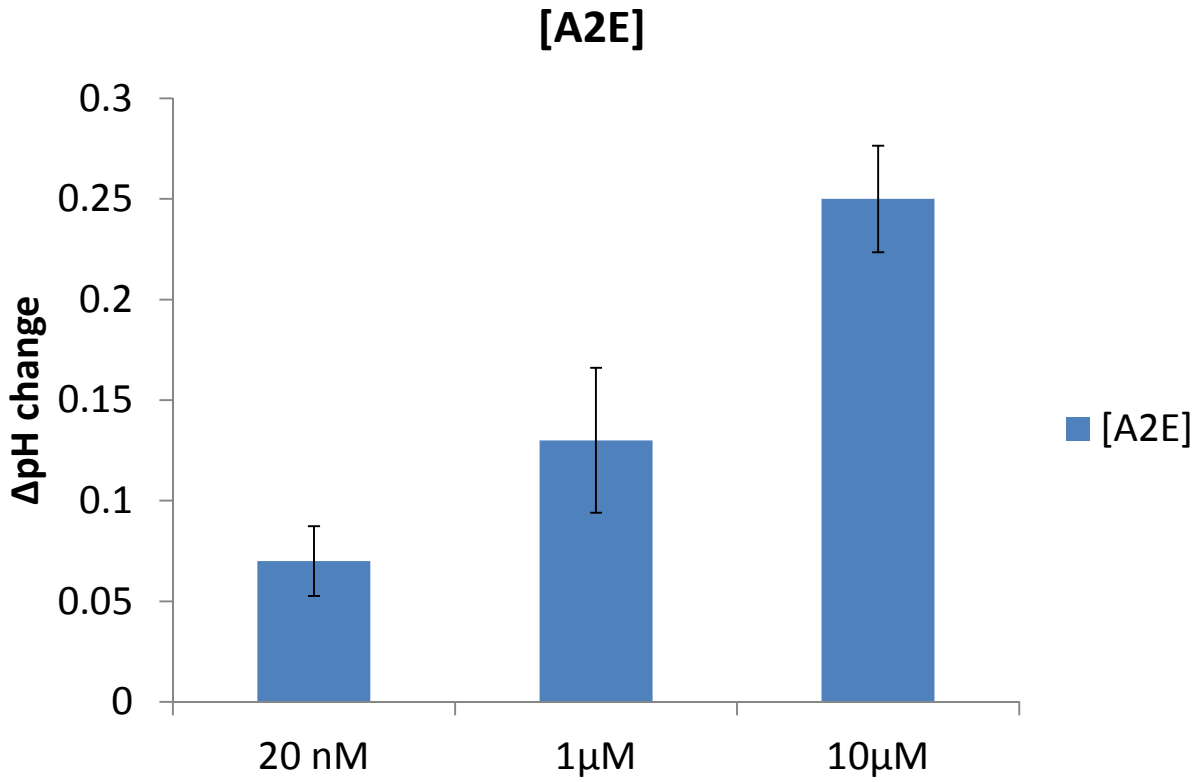


Treatment with positive control agents increases pH of ARPE-19 cells



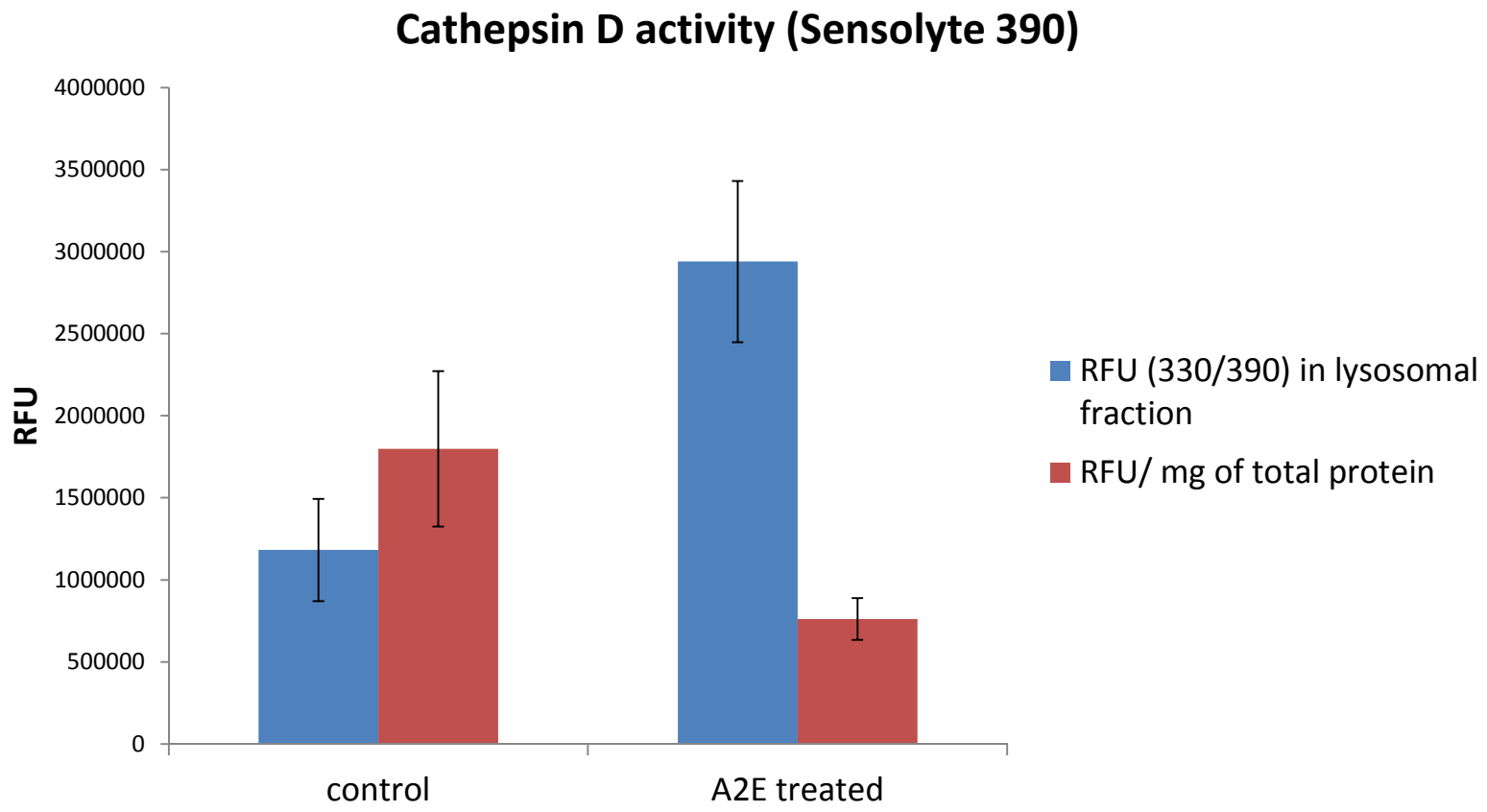
ARPE-19 confluent cells were treated for 16 hours with CQ (chloroquine), or for 4 hours with Bafilomycin A (BfA)

Effect of multiple A2E feeding of ARPE-19 cells on their lysosomal pH levels

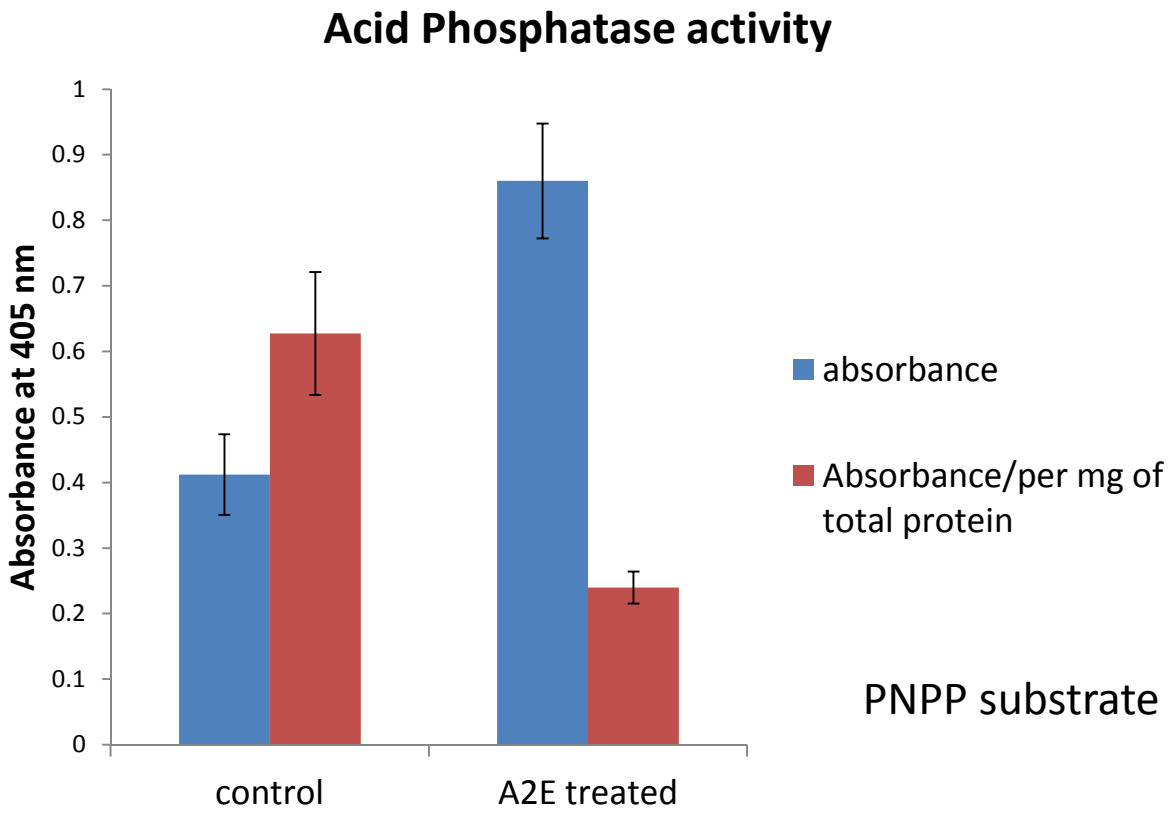


Multiple 1 μM A2E treatment leads to ~0.13 unit pH change, while treatment with10 μM leads to ~0.25 unit pH change (lysosensor DND 160 detection).

Effect of multiple A2E feeding on Cathepsin D activity in lysosomal fraction of ARPE-19 cells



Effect of multiple A2E feeding on Acid Phosphatase activity in lysosomal fraction of ARPE-19 cells



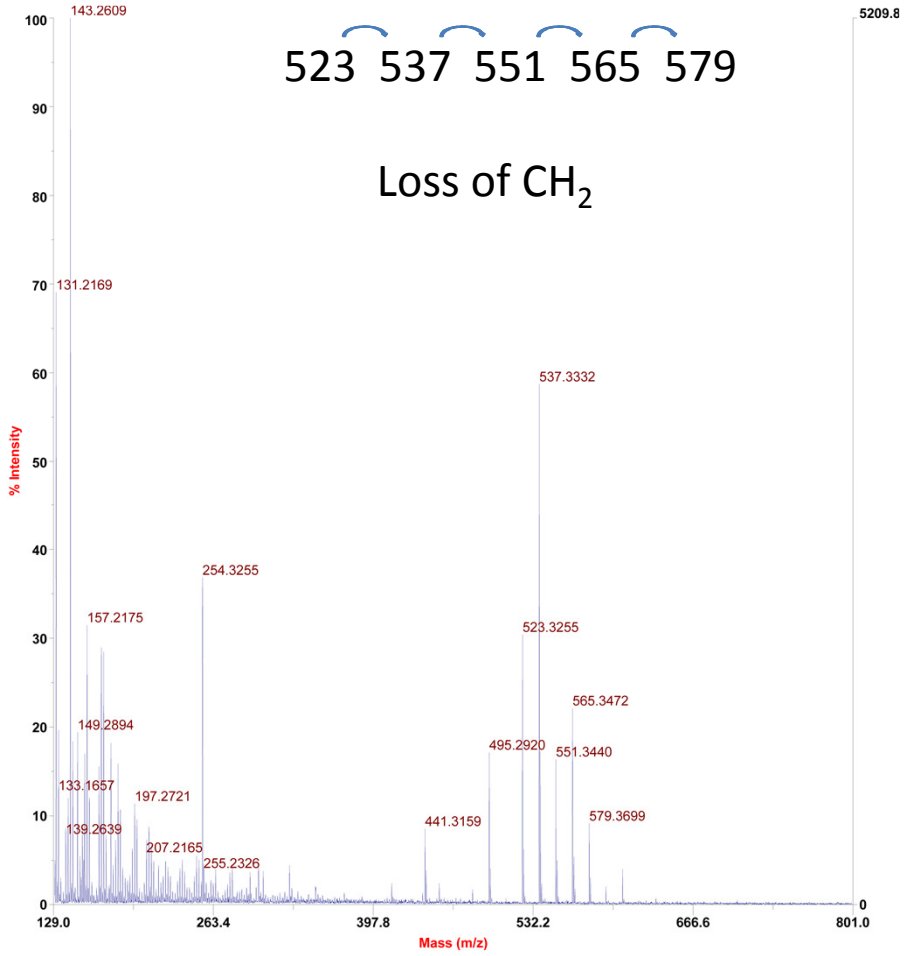
A2E accumulation leads to melanization of ARPE-19 cells upon incubation with OS

When ARPE-19 cells were incubated with isolated OS for 6 hours, followed by 18 hours chase with new media, only A2E-treated cells became pigmented.

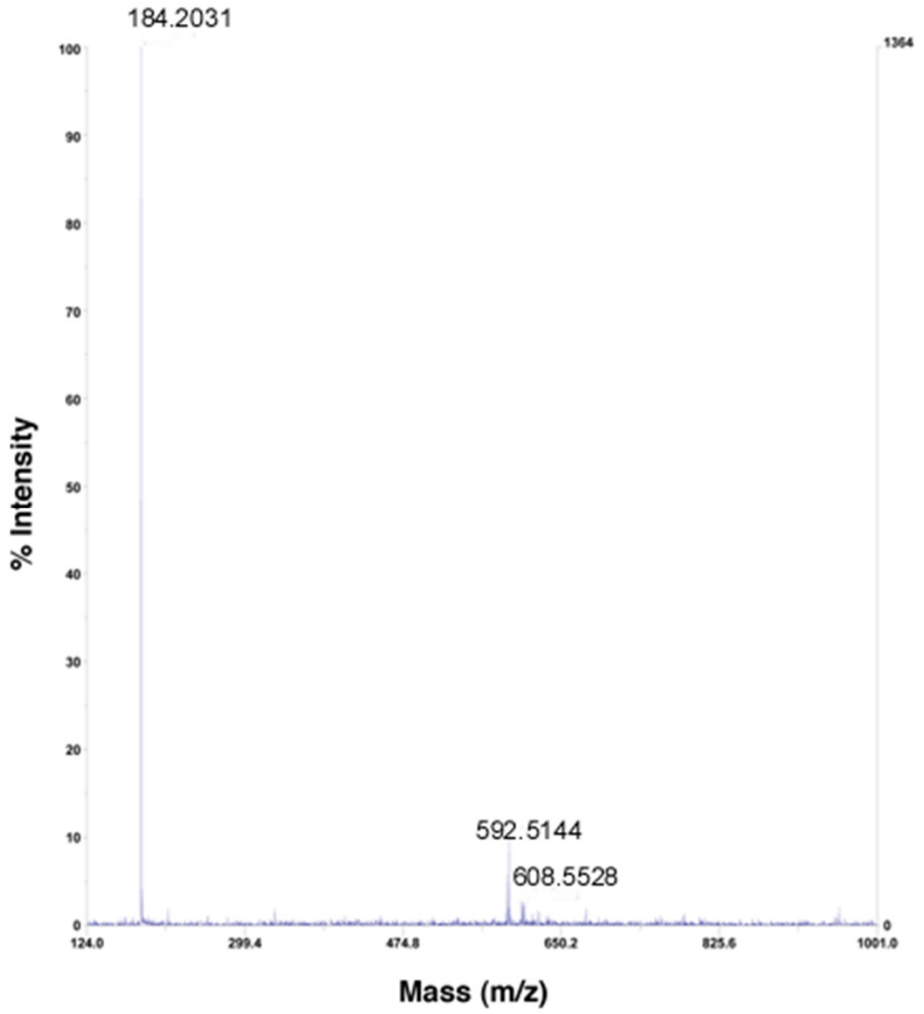
+14 +14 +14 +14

523 537 551 565 579

Loss of CH_2

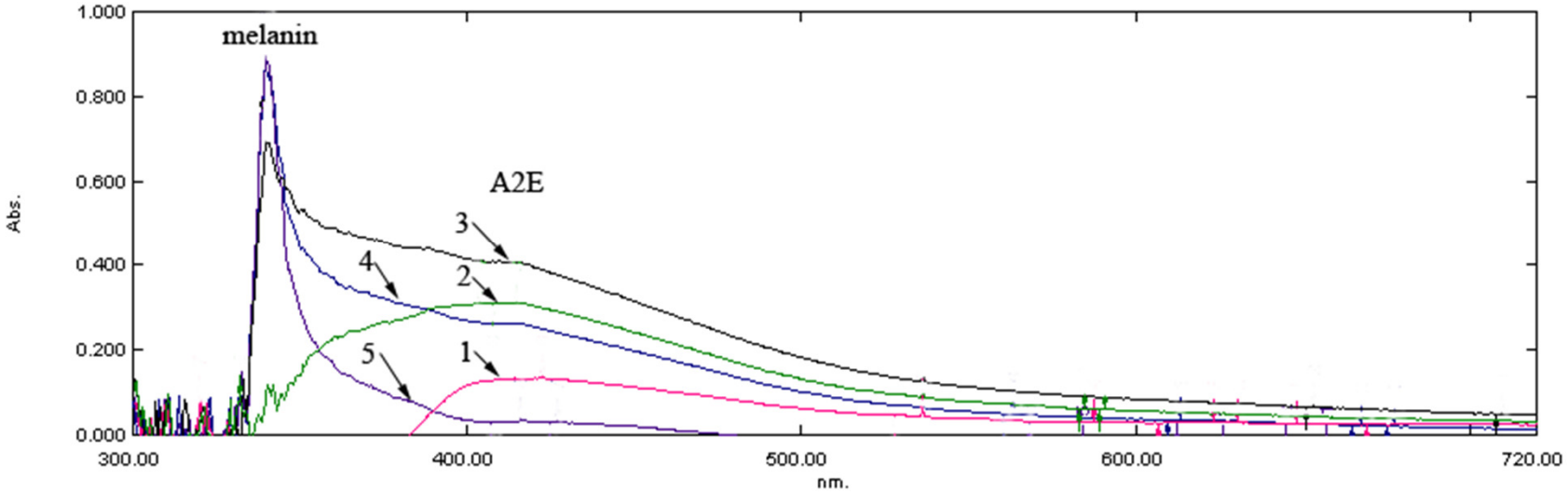


Detection of A2E in lysosomal fractions of ARPE-19 cells by mass spectrometry

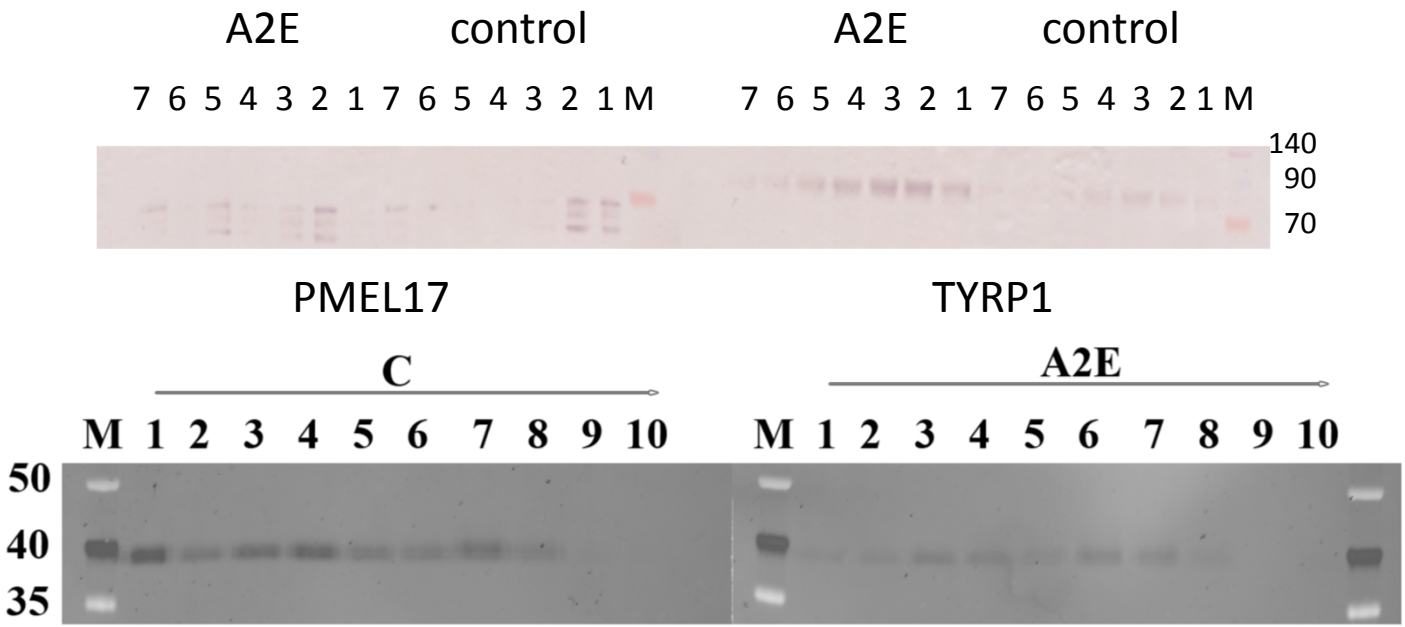


Spectral analysis of melanin and A2E in lysosomal fractions of ARPE-19 cells

Fractions 1-4 contained most of A2E and fractions 3-5 contained most of melanin. We could detect presence of melanin in A2E-containing lysosomal fractions.



Clearance of OS from ARPE-19 cells is similar in A2E-treated and control cells



Lysosomal fractions were probed with anti-Rhodopsin D4 monoclonal Ab. We see more rhodopsin in control cells but OS degradation is not impaired in A2E-treated pigmented cells.

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Conclusions

- We have developed a physiological model for aging RPE (post-confluent ARPE-19 cells, multiple treatment with non-proapoptotic concentration of A2E, OS challenge).
- A2E in our system led to mild alkalinization of lysosomes and impaired specific catalytic activity of lysosomal enzymes.
- However, ARPE-19 cells are able to compensate for the lysosome alkalinizing effect of A2E by production of melanin/melanolysosomes.
- OS clearance in pigmented A2E treated ARPE19 cells is not impaired.

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