PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE **184**, 98–101 (1987)

# Dietary Restriction Retards Age-Related Decrease in Cell Population of Rat Corneal Endothelium (42451)

MATHEW J. NADAKAVUKAREN,* KENNETH L. FITCH,* AND ARLAN RICHARDSON†

*Departments of *Biological Sciences and †Chemistry, Illinois State University, Normal, Illinois 61761*

*Abstract.* The surface areas of corneal endothelial cells from 12- and 18-month-old male Fischer 344 rats fed *ad libitum* or a calorie-restricted diet were compared. The rats fed the restricted diet in both age groups showed a statistically significant reduction in the mean cell area of the corneal endothelium. The data indicate that dietary restriction retarded the age-related endothelial cell loss and the subsequent enlargement that takes place to compensate for cell loss. This is the first report to suggest that dietary restriction retards age-related cell loss. © 1987 Society for Experimental Biology and Medicine.

The mammalian corneal endothelium, which covers the posterior surface of the cornea, is a single layer of cells that is thin and inconspicuous in cross-section and appears as a regular mosaic of hexagonal cells in flat preparations. Despite its thinness, the endothelium is an essential structure for the maintenance of normal deturgescence and transparency of the cornea (1).

Observations of the human corneal endothelium by means of the specular microscope has led to general agreement that endothelial cell density decreases with age, and that there is a corresponding increase in the mean cell area of surviving cells (2–4). As a result the aging cornea shows increasing variation in cell size and cell shape with progressively fewer hexagonal cells (5–6). Recent reports have suggested that cell division occurs in the traumatized human corneal endothelium (7–9). However, cytofluorometric nuclear DNA determinations of unwounded corneas showed most endothelial cells are of post-mitotic $G_1$ populations but that a few are in the generative $G_0$ phase, and no nuclei of S phase were found (10); thymidine incorporation has failed to show DNA replication during organ culture in the unwounded cornea (11). It remains uncertain, then, as to the role and importance of cell division in the aging, physiological human cornea. In the rabbit extensive cell division has been shown at the margin of wounds induced by mechanical denudation (12, 13), freezing (14–16), alkali chemicals (17), and acetic acid irrigation (18). However, several of these investigators (13, 14, 16) concluded that rabbit corneal endothelial cells do not divide unless provoked by a stimulus such as injury, and microfluorometric and autoradiographic analyses of noninjured tissue showed diploid amounts of DNA, leading to the conclusion that uninjured cells compose a pure $G_1$ population (16). Although endothelial mitosis has been observed in the rat 48 hr post injury, it was concluded that this animal is among mammals studied in which endothelial cells do not divide unless injured (19). Thus, we conclude that the corneal endothelial cells of rats, in the absence of trauma, behave as postreplicative cells.

In recent studies utilizing scanning electron microscopy, we reported that mean areas of endothelial cell population of female Sprague-Dawley rats aged 6, 14, and 30 months were 251, 336, and 405 $\mu m^2$, respectively (20), and those of female C57 BL/6J mice aged 1, 4, 11, 22, and 27 months were 304, 386, 458, 499, and 653 $\mu m^2$, respectively (21). The decline in cell density in rats and mice is consistent with that observed in human subjects (2–4).

Dietary restriction is the only experimental manipulation known to increase the lifespan of mammals (22); the mean and maximum survival time of rodents can be increased 25 to 40%. Dietary restriction also retards and reduces the age-related increase and incidence of a variety of diseases (23). Although the effect of dietary restriction on longevity has been well documented, the underlying mechanism of action is unknown. We examined the effect of



0037-9727/87 $1.50

dietary restriction on the surface area of corneal endothelial cells in an attempt to determine if dietary restriction has any effect on the age-related change in cell size and density in this tissue.

**Materials and Methods.** Male Fischer 344 rats, obtained from Harlan Industries were placed on two diets at 6 weeks of age. One group (control) was fed a semisynthetic diet (23) *ad libitum*, and the second group (dietary restricted) was fed daily 60% of the diet consumed by the control rats. This restriction procedure has been shown to increase the mean and maximum survival of male Fischer 344 rats by more than 40% (23). The individually caged rats were maintained on a 12-hr light–dark photoperiod under constant temperature and humidity in a barrier facility at the V.A. Medical Center (St. Louis, MO).

Eyes were removed from 12- and 18-month-old rats after decapitation, and the corneas were isolated in 0.1*M* phosphate-buffered (pH 7.2) 5% glutaraldehyde. The central portion of the corneas from the control and the restricted animals were cut into four pieces and processed simultaneously for scanning electron microscopy. After fixation in glutaraldehyde for 30 min, the corneal pieces were rinsed five times with phosphate buffer, postfixed in phosphate-buffered 1% osmium tetroxide for 30 min, dehydrated through a graded series of acetone, critical-point dried using liquid carbon dioxide, and coated with a 20-nm layer of gold. All samples were photographed at 1000X magnification. The portion to be photographed was randomly selected from each cornea. Endothelial cells from the center of the photographs were used for area calculations. Cells to be measured were numbered to avoid repeated measurements of the same cell. Cell areas were determined by tracing the cell outlines on the polaroid photographs, using a Zeiss Video Plan II digitizer and image analysis system. Both eyes from five animals in each group were used in the study for a total of 100 cells from each group. The data were statistically analyzed using a three-way analysis of variance, treatment and age as main effects, and animals as nested within treatment and age combination. Further examinations of the means were carried out using the *t* test (LSD) whenever appropriate.

**Results and Discussion.** A statistically significant difference ($P < 0.01$) was found in the mean cell areas between the two 12-month groups: the cell area of the restricted group, 368.6 ± 7.4 $\mu m^2$ (mean ± SEM), was smaller than that of the control group, 439.6 ± 6.7 $\mu m^2$. The statistical difference was even greater ($P < 0.001$) in the mean cell areas between the two 18-month groups: the cell area of the restricted group, 344.1 ± 4.7 $\mu m^2$, was smaller than that of the control group, 428.5 ± 8.5 $\mu m^2$. The frequency distributions of the cell areas in each age group show a definite shift to the smaller size in the restricted groups (Figs. 1 and 2). Thus, dietary restriction retards the age-related increase in endothelial cell size, most probably reflecting a slower rate of cell loss in the restricted animals. To the best of our knowledge, this is the first report to suggest that dietary restriction retards age-related cell loss.

It is worth noting that the differences in cell areas between restricted and control animals are greater at 18 months than at 12 months,

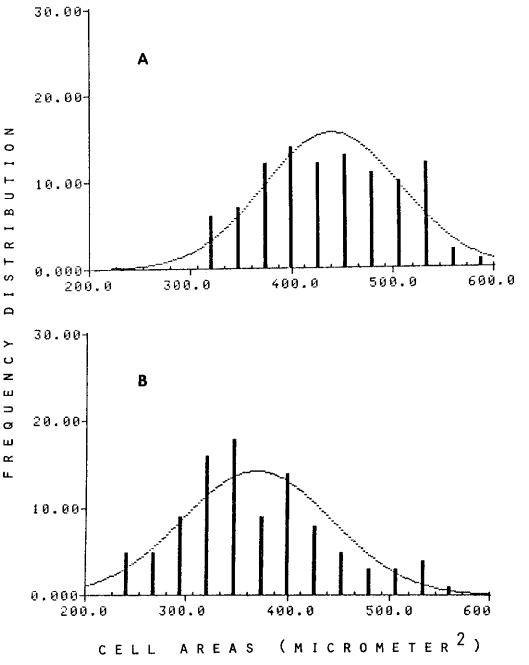


FIG. 1. Frequency distribution of corneal endothelial cell areas from 12-month-old Fischer 344 rats. (A) control group; (B) diet-restricted group. One hundred cell areas from each group are divided among a maximum of 15 size classes. The continuous curve represents the computer-generated Gaussian distribution.

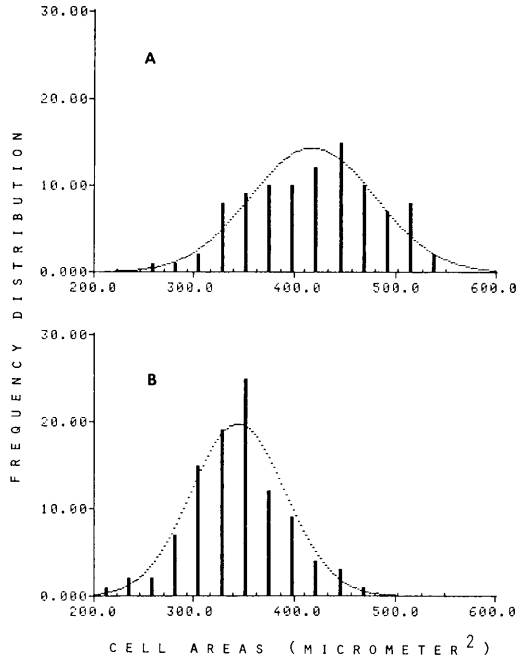


FIG. 2. Frequency distributions of corneal endothelial cell areas from 18-month-old Fischer 344 rats. (A) control group; (B) diet-restricted group. One hundred cell areas from each group are divided among a maximum of 15 size classes. The continuous curve represents the computer-generated Gaussian distribution.

suggesting a possible increased effect in animals maintained on restricted diet for longer periods of time.

It has been reported previously that dietary restriction can decelerate age-related loss of soluble $\gamma$ crystallins in the mouse lens (24). In man, loss of $\gamma$ crystallins also occurs with age, and is associated with cataract development (25). It is not surprising that these two ocular structures, the lens and the corneal endothelium, show parallel age-related changes, because both are avascular and dependent upon the aqueous fluid for nourishment (26). Any change in the composition of the aqueous fluid or its rate of formation might be expected to influence the metabolism of both structures. These data support the suggestion that animals on dietary restriction can be useful experimental models for studying the aging process in the eye, and also indicate that the corneal endothelium can be an index for the estimation of physiological age in experimental animals.

We thank Dr. Herman Brockman and Dr. George Kidder III for the internal review of the manuscript and for providing helpful suggestions. We also thank Dr. Ramesh Chaudhari and Dr. Alan Katz for assistance in the statistical analysis of data. This project is supported by NIH Grant AG00344.

1. Waltman SR. In: Moses, RA, Ed. Adler's Physiology of the Eye: The Cornea. St. Louis, Mosby, 7th ed. p48, 1981.
2. Bourne WM, Kaufman HE. Specular microscopy of human corneal endothelium in vivo. Amer J Ophthalmol **81**:319–323, 1976.
3. Laing RA, Sandstrom MM, Berrospi AR, Leibowitz HM. Changes in the corneal endothelium as a function of age. Exp Eye Res **22**:587–594, 1976.
4. Laule A, Cable MK, Hoffman CE, Hanna C. Endothelial cell population changes of human cornea during life. Arch Ophthalmol **96**:2031–2035, 1978.
5. Shaw EL, Rao GN, Arthur EJ, Aquavella JV. The functional reserve of the corneal endothelium. Ophthalmology **85**:640–649, 1978.
6. Suda T. Mosaic pattern changes in the human corneal endothelium with age. Japan J Ophthalmol **28**:331–338, 1984.
7. Treffers WF. Human corneal endothelial wound repair, in vitro and in vivo. Ophthalmology **89**:605–613, 1982.
8. Laing RA, Neubauer L, Setsuko S, Oak BA, Kayne HL, Leibowitz HM. Evidence for mitosis in the adult corneal endothelium. Ophthalmology **91**:1129–1134, 1984.
9. Olsen EG, Davanger M. The healing of human corneal endothelium: An in vitro study. Acta Ophthalmol (Copenhagen) **62**:885–892, 1984.
10. Ikebe H, Takamatsu T, Itoi M, Fujita S. Cytofluorometric nuclear DNA determinations on human corneal endothelial cells. Exp Eye Res **39**:497–504, 1984.
11. Simonsen AH, Sorensen KE, Sperling S. Thymidine incorporation by human corneal endothelium during organ culture. Acta Ophthalmol (Copenhagen) **59**: 110–118, 1981.
12. Binder RF, Binder HF. Regenerative processes in the endothelium of the cornea. Arch Ophthalmol **57**:11–13, 1975.
13. Chi C, Teng CC, Katzin HM. Healing process in the mechanical denudation of the corneal endothelium. Amer J Ophthalmol **49**:693–703, 1960.
14. Faure JP, Kim YZ, Graf B. Formation of giant cells in the corneal endothelium during its regeneration after destruction by freezing. Exp Eye Res **12**:6–12, 1974.
15. Van Horn DL, Sendele DD, Seideman S, Buco PJ. Regenerative capacity of the corneal endothelium in rabbit and cat. Invest Ophthalmol Vis Sci **16**:597–613, 1977.
16. Gordon SR, Rothstein H. Studies on corneal endo-

thelial growth and repair. I. Microfluorometric and autoradiographic analyses of DNA synthesis, mitosis and amitosis following freeze injury. Metabol Ophthalmol **2**:57–63, 1978.
17. Matsuda H, Smelser GK. Endothelial cells in alkali-burned corneas: Ultrastructure alterations. Arch Ophthalmol **89**:402–409, 1973.
18. Stoneburner S, Thresher R, Cobo LM, Klintworth GK. Endothelial cell repopulation after intracameral acetic acid irrigation. Graefe's Arch Clin Exp Ophthalmol **223**:139–144, 1985.
19. Gordon SR, Rothstein H, Harding CV. Studies on corneal endothelial growth and repair. IV. Changes in the surface during cell division as revealed by scanning electron microscopy. Eur J Cell Biol **31**:26–33, 1983.
20. Fitch KL, Nadakavukaren MJ, Richardson A. Age-related changes in the corneal endothelium of the rat. Exp Gerontol **17**:179–183, 1982.
21. Fitch KL, Nadakavukaren MJ. Age-related changes in the corneal endothelium of the mouse. Exp Gerontol **21**:31–36, 1986.
22. Ross MH. Dietary behavior and longevity. Nutr Rev **35**:257–265, 1977.
23. Yu BP, Masoro EJ, Murata I, Bertrand HA, Lynd FT. Life span study of SPF Fischer 344 male rats fed *ad libitum* or restricted diets: Longevity, growth, lean body mass and disease. J Gerontol **37**:130–141, 1982.
24. Leveille PJ, Weindruch R, Walford RL, Bok D, Horwitz J. Dietary restriction retards age-related loss of gamma crystallins in the mouse lens. Science **224**: 1247–2249, 1984.
25. Charlton JM, van Heyningen R. An investigation into the loss of proteins of low molecular size from the lens in senile cataract. Exp Eye Res **7**:47–55, 1968.
26. Sears ML. In: Moses RA, Ed. Adler's Physiology of the Eye: The Aqueous. St. Louis, Mosby, 7th ed. p204, 1981.

---

Received December 30, 1985. P.S.E.B.M. 1987, Vol. 184.
Accepted October 2, 1986.