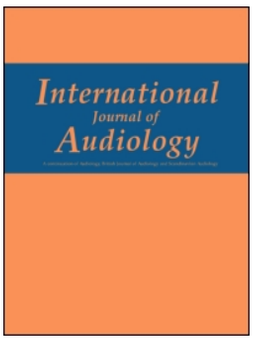

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Dietary Restriction and Presbyacusis: Periods of Restriction and Auditory Threshold Losses in the CBA/J Mouse

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Key Words. Presbyacusis · Diet · Mouse · Aging · Audition

Abstract. Dietary restriction was imposed on CBA/J mice, animals which develop presbyacusis late in their lives. Animals restricted for their whole lives, as well as those restricted after midlife, had less presbyacusis than did control mice fed ad libitum. Dietary restriction did not increase the life spans of these mice. Restriction until midlife did not protect from presbyacusis, nor did it increase life span. In this genotype, dietary restriction protects against hearing loss only if it occurs at the age of most rapid decline of cochlear function.

Introduction

A major desire of individuals in our society is to extend the quality and span of their lives. In experimental animals (primarily rodents), the most reliable and effective means of prolonging life has been through dietary restriction [Walford, 1983; Yu, 1985]. Current interest in improving nutritional standards in our society suggests that we have similar expectations concerning our life spans. Many of the persons who are becoming nutrition conscious are currently middle aged. Therefore the finding that rodents can still benefit from restriction beginning in midlife is of some interest [Weindruch et al., 1982; Stuchlikova et al., 1975].

Dietary restriction initiated at weaning has been known to increase median and maximum life span since the early studies of McCay et al. [1935]. Most subsequent studies have focused on the retardation of age-related physiological deterioration and disease processes which might explain the life span extension [see Yu, 1987, 1985 for reviews], without questioning whether the quality of life is maintained during the extended period. One inevitable consequence of aging which disrupts the quality of many human lives is the loss of hearing. Dietary restriction beginning at weaning has previously been shown to attenuate hearing loss in one inbred strain of mice [Henry, 1986]. The present study assessed auditory func-

tion in a different strain of mice whose diets had been restricted for either the first half or second half of their lives.

Human presbyacusis is characterized by a progressive high-frequency loss that is associated with loss of hair cells at the basal turn of the cochlea [Schuknecht, 1955]. Presbyacusis also involves familial factors such as genetics [Paparella et al., 1975], and a good animal model should account for these characteristics. The aging mouse appears to show peripheral changes that are very similar to those that occur in humans. Behavioral, physiological, and anatomical studies have shown the high-frequency threshold elevations and basal hair cells losses that characterize human presbyacusis [Ralls, 1967; Mikaelian et al., 1974; Ehret, 1974; Henry and Chole, 1980; Henry, 1982]. Many different strains of mice have had their age-related hearing losses well documented [see Henry, 1983 for a review] providing a genetic base for studying presbyacusis in this species. Among these strains, the CBA/J best approximates the time course of the human condition, suffering the brunt of its high-frequency losses after midlife [Henry and Chole, 1980].

Recent experiments have begun to study the effects of dietary restriction during shorter portions of the animal's life span [Weindruch et al., 1982; Cheney et al., 1983; Masoro, 1984]. The results of these studies suggest that whole-life dietary restriction initiated at weaning is still the most effective means of extending life span. However, they also show that restriction for shorter periods of time is also able to prolong life [see Yu, 1985, 1987 for reviews]. The present study was designed to assess the effects of dietary restrictions at different life stages on auditory function in the aging mouse.

Methods

This experiment used second-to sixth-generation descendants of CBA/J parental stock obtained from the Jackson Laboratory. All of these highly inbred mice were born and reared in our colony under conditions of restricted ambient noise and remained drug-free until testing. The CBA/J is characterized as a normal-hearing, long-lived mouse (mean life span > 800 days; Harrison, 1982]. It develops a gradual sensorineural hearing loss in the last half of its life [Henry and Chole, 1980]. This hearing loss is most prominent at higher frequencies and is thus similar in pattern to human age-related hearing loss.

Sixty-seven mice were assigned to three experimental and two control condition. The experimental conditions of dietary restriction were produced by a gradual reduction (over 2 weeks) of access to food (Simonsen lab chow). At the end of this period, the mice were only fed on Monday, Wednesday, and Friday. Care was taken to insure that no food remained in their cages on the other 4 days of the week. A whole-life (WL; n = 13) experimental group was restricted from weaning until death; a first-half (FH; n = 13) experimental group was restricted from weaning until midlife (300 days); and a second-half (SH; n = 15) experimental group was restricted from midlife until death. Both control groups were fed ad libitum throughout their lives. The first control group (n = 15) consisted of littermates of the SH group, and both these groups were assigned from the colony at midlife. The second control group (n = 12) was used for comparison with the WL and FH groups at the first test date (300 days). Mice in our colony were screened for otitis and no cases were reported for CBA/J mice during the course of this study.

Auditory function was assessed by obtaining auditory brainstem response (ABR) thresholds from the left ear of each mouse in response to frequencies of 4, 8, 16, 32, and 64 kHz. Prior to testing at 300 days, all mice were anesthetized with pentobarbital (75 mg/kg i.p.) and given an equivalent amount of atropine sulfate to reduce respiratory distress. At the 500- and 800-day test dates animals were pretranquilized with chlorprothixine (5 mg/kg, i.m.) and subsequently anesthetized with ketamine (150 mg/kg, i.p.). The anesthetic was changed due to poor survival rates obtained when using pentobarbital. Body temperature was actively maintained between 36 and 39 °C [mouse ABR thresholds remain stable over this range; Henry,

1980]. Each mouse was tested in a sound-attenuated chamber that was electrically and magnetically shielded and grounded.

The ABR was nontraumatically obtained via stainless steel electrodes situated at the vertex and soft palate. A pair of cascading preamplifiers adjacent to the mouse provided a signal gain of 100 000, and the ABR was then actively filtered (300–5 000 Hz, 24 dB/octave roll-off) by equipment which was outside of the chamber. The filtered bioelectric signals were processed with a Nicolet 1170 signal averager equipped with an artifact rejection module.

Acoustic stimuli were shaped and gated (1 ms duration, 300 μs rise and decay time for 16–64 kHz; 2 ms duration, 600 μs rise and decay time for 4 and 8 kHz) by logic modules. The tone bursts were presented at a rate of 20/s, transduced by either a 1-inch Bruël & Kjaer microphone rewired as a speaker (16–64 kHz) or a Yamaha piezoelectric headphone (4 and 8 kHz). The ceiling for sound intensity was 80 dB SPL at all frequencies. Quasi-free-field stimulation was used, and a Bruël & Kjaer microphone (¼ inch) was positioned at the tragus to measure SPL. The output of this microphone was also monitored on an oscilloscope to insure that the stimuli were distortion free during the course of the experiment. Periodically, the acoustic stimuli were analyzed more thoroughly with a Hewlett-Packard 3561A signal analyzer.

Mice were initially stimulated at each frequency at SPLs between 65 and 80 dB. If a clear ABR was reliably obtained, the stimulus output was attenuated in 10-dB steps until the response was no longer clearly visible, at which time the intensity was increased by 5 dB and the threshold was extrapolated visually. Differences in thresholds between the groups were subjected to an analysis of variance at each frequency, and subsequent evaluations were based on the results of two-tailed t tests between groups. The second control group tested for thresholds at 300 days was not utilized in any other analyses.

The primary cause of death of animals in this study was due to the anesthetic used during testing; animals that died in this manner were not included in the life span analysis. All four groups were equally affected by the drug-related deaths. Life span data for four of the groups (3 experimental, 1 control) were subjected to an analysis of variance. Post hoc contrasts provided information about the age differences between any two of the individual groups. Differences in mean weight were expected a priori between the control and restricted groups; therefore, individual t tests were used to describe weight differences between the experimental and control animals.

Results

Dietary restriction did not increase the life span of the three experimental groups of CBA/J mice; in fact, restriction was associated with a shorter life span in two of these groups. The three regimens of dietary restriction and the control diet in this study differed significantly in their effects upon the mean life span of the CBA/J mouse ($F=7.97$; $p<0.001$). The means and standard errors for life span of the four groups, as well as the t values comparing the three experimental groups with the control group, are presented in table I. Post hoc analyses also determined that there was no significant difference between the WL and SH animals ($t=0.353$; $p>0.05$), and that individually these two groups were significantly different from the group ($t=8.61$, $p<0.01$, WL; $t=10.19$, $p<0.01$, SH). The life span of the FH mice did not significantly differ from that of the control mice ($t=0.32$, $p>0.05$). Therefore dietary restriction during the first half of life does not affect life span in the CBA/J mouse. The mean

Table I. Mean life span and t values (vs. control animals) for the three experimental groups

Group	Life span mean ± SEM, days	t value (vs. control)
Control	864.1 ± 20.9	
SH	644.3 ± 62.3	t = 14.9*
FH	854.6 ± 30.4	t = 0.22
WL	675.9 ± 35.9	t = 13.5*

*$p<0.001$.

Table II. Mean weights and t values (vs. control) at three test ages

Age	Group	Weight, g mean ± SEM	t values vs. control
	Control	31.1 ± 0.66	
300 days	SH	31.4 ± 0.66	0.28
	FH	24.3 ± 0.75	6.31**
	WL	26.1 ± 0.71	4.98**
	Control	29.9 ± 1.1	
500 days	SH	27.6 ± 1.7	1.22
	FH	29.4 ± 1.4	0.32
	WL	26.6 ± 0.86	2.20*
	Control	29.8 ± 1.2	
800 days	SH	26.4 ± 4.0	1.26
	FH	32.5 ± 0.5	1.04
	WL	no data	no data

*$p < 0.05$; **$p < 0.001$.

weights of the animals in the four groups at each test date as well as t values (vs. control) are presented in table II. In general, animals that had ad libitum access to food at the time of testing were significantly heavier than their restricted counterparts.

The three schedules of dietary restriction also had different effects on the auditory function of CBA/J mice. Life span auditory thresholds are presented for three different ages in figures 1–3. At 300 days (fig. 1), all groups had nearly the same auditory thresholds at frequencies up through 32 kHz. At 64 kHz, the control animals showed a significant slight elevation ($F = 11.9$, $p < 0.01$).

At 500 days, WL animals had thresholds which were only slightly elevated compared to their thresholds at 300 days. At this age, SH animals showed slightly higher threshold elevations but none which were significantly different from 300-day control animals. FH animals at 500 days were not different from WL or SH animals at frequencies of 16 kHz and below. However, the FH thresholds were significantly higher than those of those two groups at frequencies of 32 and 64 kHz ($t = 6.04$, $p = < 0.01$; $t = 6.88$, $p > 0.01$). Control animals at this age had highly significant elevations at all frequencies.

By 800 days, both the control animals and the FH animals had developed significant threshold elevations at all frequencies, but the SH animals had thresholds which were less severely elevated at frequencies below 64 kHz. All of the WL animals died of natural causes before the 800-day test date. Flat audiograms in older animals which might suggest conductive losses, are actually a consequence of statistical averaging and the sound level ceiling. Individual audiograms did not show the conductive loss profile.

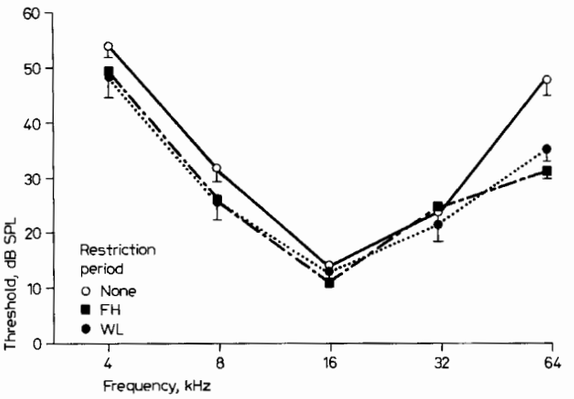

Fig. 1. The mean (-1 SE) ABR threshold values for two experimental groups (WL, n=10; FH, n=10) restricted from weaning versus controls (n=12) fed ad libitum at 300 days. The three groups are not significantly different below 64 kHz. At 64 kHz, the control animals have a significant threshold elevation.

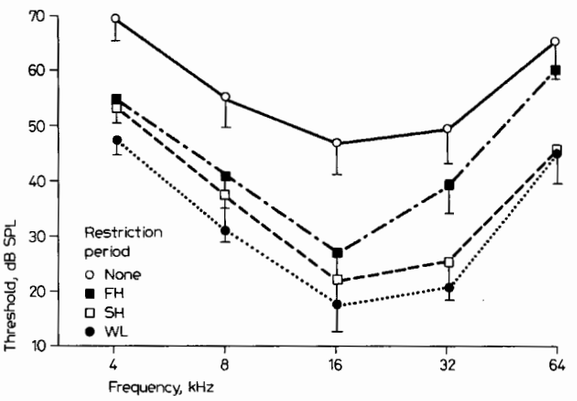

Fig. 2. Mean (-1 SE) ABR threshold values from four groups at 500 days. The WL group (n=8) maintained thresholds which are similar to their 300-day values. The SH group (n=7) had mean threshold values which are not significantly different from the WL group at this age, and similar to the control animals at 300 days. FH animals (n=8) had threshold elevations at all frequencies compared to their 300-day values, these elevations reached significance at 32 and 64 kHz. The control animals (n=12) had significant elevations at all frequencies compared to both their 300-day values and values for the SH and WL groups at 500 days.

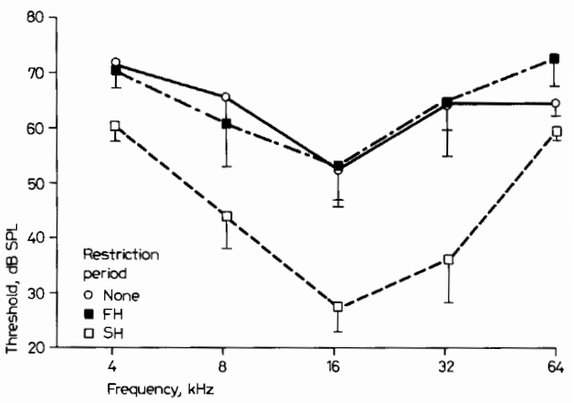

Fig. 3. Mean ABR threshold values for two experimental and one control group at 800 days. The FH (n=7) and control (n=9) animals show large threshold elevations characteristic of presbyacusic mice. The SH group (n=2) shows threshold elevations relative to earlier test dates, but these losses are less severe. The SH animals have been on restricted diets for 500 days at this test date and this period covers the time in which most presbyacusic changes occur in this strain.

Discussion

The three schedules of dietary restriction had different effects on the life spans of the CBA/J mice in this study. Both groups that were restricted during the second half of their lives (WL and SH mice) lived significantly shorter lives than the control animals, whose life spans were similar to the actuarial values reported for this genotype [Harrison, 1982]. The FH animals attained their actuarial life span, but did not have extended lives. The decreased life span in two of our experimental groups might suggest that our regimen of dietary restriction was too severe. However, it could be that the CBA/J mouse is one of the few strains of rodent that does not attain an extended life when restricted in diet. Harrison and Archer [1987] and Kokkonen and Barrows [1985] have reported cases of decreased life span with different forms of dietary restriction in the C57BL/6 mouse. The percentage of drug-related deaths does not allow for an unequivocal statement concerning life span in this study.

The time course of presbyacusis was also affected differently by the various periods of dietary restriction used in this study. As they aged, animals that were restricted in the second half of their lives (WL and SH animals) did not lose their auditory function as fast as the control and FH animals. At 500 days the SH and WL animals had thresholds that were similar to those of 300-day control animals. The control animals at 500 days had significant elevations at all frequencies, and the FH animals showed the high-frequency elevations which characterize the onset of presbyacusis in the mouse (and human). The WL animals in the present study showed reductions in presbyacusic changes similar to those of restricted AU/Ss mice from an earlier study [Henry, 1986].

The present study is not the first to report negative findings for dietary restriction and presbyacusis. Feldman [1984] described anatomical changes in the cochleae of rats whose lives were extended by dietary restriction that were not different from those of control animals. His finding is similar to ours: FH animals had auditory threshold losses similar to those of control animals. Dietary restriction has a different effect on the AKR/J inbred mouse: it affects neither presbyacusis nor life span [Henry, 1986]. In sum, dietary restriction can have a variety of effects, depending upon the period and the genotype: it can extend life span and retard presbyacusis [Henry, 1986], it can decrease life span and retard presbyacusis (present study), it can increase life span without influencing presbyacusis [Feldman, 1984], and it can also have no effect on either presbyacusis or life span [Henry, 1986]. The nonuniform effects of dietary restriction on presbyacusis in this study have suggested a hypothesis to explain these and possibly other related data.

Our data suggest that *dietary restriction may only attenuate an age-related loss if the restriction covers the period when this decline normally occurs.* The CBA/J mouse is characterized by its presbyacusic changes which occur after midlife [Henry and Chole, 1980]. This would suggest that FH animals did not decrease their auditory losses because the dietary restriction did not occur during the time period when presbyacusis is most prevalent in this strain. To verify or disprove this suggestion, more data from different animals and different biological systems are required. In order to address this hypothesis, we are currently studying pres-

byacusic changes in other strains of mice whose periods of auditory vulnerability are different from those of the CBA/J.

The variable effects of dietary restriction reported in this and other studies [Harrison and Archer, 1987; Kokkonen and Barrows, 1985; Feldman, 1984] would suggest that dietary restriction may not be the panacea that many humans desire. It may or may not increase life span, and even if it does, an extended quality of life is not necessarily guaranteed. The extended life span may be one of silence or reduced neuromuscular ability. The present data do not allow us to predict the outcome of a human dietary restriction regimen. Perhaps the greatest value of the present study is that it represents a step towards isolating the effects of dietary restriction on those genes which influence presbyacusis in mice.

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Restrictions alimentaires et presbyacousie: la période de restriction affecte les diminutions du seuil auditif chez la souris CBA/J de façon différentielle

On a imposé une restriction alimentaire à des souris CBA/J, animaux chez lesquels la presbyacousie apparaît à un âge avancé. Les animaux sous restriction alimentaire, soit durant leur vie entière, soit durant la seconde moitié de leur vie seulement, on manifesté moins de (symptômes de) presbyacousie que les animaux non soumis à de telles restrictions. Cependant, ces contraintes alimentaires ont également diminué la longévité de vie des souris. Les restrictions imposées durant la première moitié de la vie n'ont ni protégé les animaux contre la presbyacousie, ni diminué leur longévité. Dans ce génotype, les restrictions alimentaires protègent contre la perte de l'ouïe, uniquement si celles-ci sont pratiquées à l'âge du déclin le plus rapide.

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