



## Research paper

## Auditory function in rhesus monkeys: Effects of aging and caloric restriction in the Wisconsin monkeys five years later

Cynthia G. Fowler<sup>a,\*</sup>, Kirstin Beach Chiasson<sup>b,1</sup>, Tami Hanson Leslie<sup>c,2</sup>, Denise Thomas<sup>d,3</sup>, T. Mark Beasley<sup>e,4</sup>, Joseph W. Kemnitz<sup>f,5</sup>, Richard Weindruch<sup>g,6</sup><sup>a</sup> Department of Communicative Disorders, University of Wisconsin–Madison, 1975 Willow Drive, Madison, WI 53706, United States<sup>b</sup> Nationwide Children's Hospital, 700 Children's Drive, Columbus, OH 43205, United States<sup>c</sup> Greater Milwaukee Otolaryngology, LLC, 4600 West Loomis Road, Suite 201, Greenfield, WI 53220, United States<sup>d</sup> Children's Memorial Hospital, 2300 Children's Plaza, Chicago, IL 60614–3363, United States<sup>e</sup> Department of Biostatistics, School of Public Health, Ryals Public Health Bldg., University of Alabama at Birmingham, Birmingham, AL 35294, United States<sup>f</sup> Wisconsin National Primate Research Center, Department of Physiology, University of Wisconsin–Madison, 1220 Capitol Court, Madison, WI 53715–1299, United States<sup>g</sup> Department of Medicine, University of Wisconsin–Madison, Room B72 Veterans Admin Hospital, William S. Middleton Memorial Veterans Hospital, 2500 Overlook Terrace, Madison, WI 53705–2286, United States

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## ABSTRACT

Caloric restriction (CR) slows aging in many species and protects some animals from age-related hearing loss (ARHL), but the effect on humans is not yet known. Because rhesus monkeys are long-lived primates that are phylogenically closer to humans than other research animals are, they provide a better model for studying the effects of CR in aging and ARHL. Subjects were from the pool of 55 rhesus monkeys aged 15–28 years who had been in the Wisconsin study on CR and aging for 8–13.5 years. Distortion product otoacoustic emissions (DPOAE) with  $f_2$  frequencies from 2211 to 8837 Hz and auditory brainstem response (ABR) thresholds from clicks and 8, 16, and 32 kHz tone bursts were obtained. DPOAE levels declined linearly at approximately 1 dB/year, but that rate doubled for the highest frequencies in the oldest monkeys. There were no interactions for diet condition or sex. ABR thresholds to clicks and tone bursts showed increases with aging. Borderline significance was shown for diet in the thresholds at 8 kHz stimuli, with monkeys on caloric restriction having lower thresholds. Because the rhesus monkeys have a maximum longevity of 40 years, the full benefits of CR may not yet be realized.

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## 1. Introduction

Age-related hearing loss (ARHL), or presbycusis, is prevalent in older humans and is a significant cause of reduced productivity, social isolation, and depression in this population (Bess et al., 1989). Presbycusis can affect all levels of the auditory system, resulting in a loss of auditory sensitivity as well as increased difficulty in

understanding speech, especially in noisy backgrounds. Alleviation or postponement of presbycusis could be a factor in increasing the quality of life in older individuals. Currently presbycusis is not preventable, but research on caloric restriction (CR) is promising. CR, defined as a reduction in calories but with adequate nutrients, has been shown to prolong lifespan and improve general health in numerous species (for a review, see Weindruch and Walford, 1988). Once the underlying mechanisms of CR are understood, a targeted approach to increasing both lifespan and health span for humans may be developed. The development of adequate animal models of aging and sensory function is crucial to obtaining these goals.

Rhesus monkeys may provide better insight into the effects that CR is likely to have on humans as compared to other laboratory animals, such as rodents. With a maximum life expectancy of approximately 40 years in captivity (Tigges et al., 1988), rhesus monkeys are a long-lived species that experience many of the same age-related diseases as humans do (Roth et al., 2004). Auditory function in the monkeys also approaches that of humans, including

*Abbreviations:* ARHL, age-related hearing loss; CR, caloric restriction; DPOAE, distortion product otoacoustic emissions

\* Corresponding author. Tel.: +1 608 262 6483; fax: +1 608 262 6466.

*E-mail addresses:* [cgfowler@wisc.edu](mailto:cgfowler@wisc.edu) (C.G. Fowler), [Kirstin.Chiasson@nationwidechildrens.org](mailto:Kirstin.Chiasson@nationwidechildrens.org) (K.B. Chiasson), [tamil@gmodocs.com](mailto:tamil@gmodocs.com) (T.H. Leslie), [DThomas@childrensmemorial.org](mailto:DThomas@childrensmemorial.org) (D. Thomas), [mbeasley@ms.soph.uab.edu](mailto:mbeasley@ms.soph.uab.edu) (T.M. Beasley), [kemnitz@primate.wisc.edu](mailto:kemnitz@primate.wisc.edu) (J.W. Kemnitz), [rhweindr@wisc.edu](mailto:rhweindr@wisc.edu) (R. Weindruch).

<sup>1</sup> Tel.: +1 614 722 3984; fax: +1 614 722 3942.

<sup>2</sup> Tel.: +1 414 281 4642.

<sup>3</sup> Tel.: +1 773 880 3174; fax: +1 773 880 6618.

<sup>4</sup> Tel.: +1 205 975 4957; fax: +1 205 975 2540.

<sup>5</sup> Tel.: +1 608 263 3588; fax: +1 608 265 8439.

<sup>6</sup> Tel.: +1 608 256 1901x11642.

middle ear (Fowler et al., 2008; Torre et al., 2000), cochlear (Lasky et al., 2002), and neural function (Fowler et al., 2002; Lasky et al., 1995).

Otoacoustic emissions and auditory brainstem responses (ABR) have been used to document ARHL in both humans and monkeys. Otoacoustic emissions decrease in level and frequency in older humans (e.g., Lonsbury-Martin et al., 1991; Uchida et al., 2008) and in older rhesus monkeys (Torre and Fowler, 2000; Torre et al., 2004). ABR thresholds increase with age in both humans (Rowe, 1978) and rhesus monkeys (Torre and Fowler, 2000). Fowler et al. (2002) reported some beneficial effects of CR in the female monkeys but not in male monkeys in the Wisconsin study of CR (Kemnitz et al., 1993). Torre et al. (2004) evaluated male monkeys in the NIA study on CR (Ingram et al., 1990; Lane et al., 1992) and found no effects of CR on auditory function. They suggested that the male monkeys were either resistant to the beneficial effects of CR or that CR was begun too late in life to be beneficial for preservation of hearing.

The University of Wisconsin study of CR in rhesus monkeys was begun in 1989 to address its effect on longevity and health span (Kemnitz et al., 1993), and the study of the effect of CR on auditory function in this population was begun in 1999 (Fowler et al., 2002). The CR monkeys are displaying improved health span and reduced mortality compared to the control monkeys (Coleman et al., 2009); CR monkeys have lower body fat, improved insulin sensitivity, and better cardiovascular function than their age-matched, ad libitum counterparts (Anderson and Weindruch, 2006; Anderson et al., 2009). The positive physiological response of the monkeys to CR reduces some of the known risk factors for hearing loss in humans, such as cardiovascular disease (Johnsson and Hawkins, 1972; Meyer et al., 2006; Rosen and Olin, 1965) and diabetes (Dalton et al., 1998; Frisina et al., 2006), thus suggesting the possibility that hearing could be preserved in the CR monkeys as they age.

The purposes of the present study were (1) to continue the development of rhesus monkeys as a model for human presbycusis by evaluating auditory function, (2) to investigate the effect of CR on auditory function associated with ARHL, and (3) to determine the duration of CR necessary to exert protective effects against ARHL. For the first two purposes, the rhesus monkeys in the University of Wisconsin study on the effects of CR and aging were evaluated with distortion product otoacoustic emissions (DPOAE) and ABR thresholds. For the final purpose, the cohort monkeys that had been in the study the longest (162 months or 13.5 years) were evaluated separately.

## 2. Methodology

### 2.1. Materials

Otosopic examinations were performed, followed by acoustic immittance measures. A middle ear analyzer (Grason-Stadler, Model 38 calibrated to ANSI, 1987 standards) was used to obtain tympanograms to ensure normal middle ear function. An auditory evoked potential system (Intelligent Hearing Systems) was used for DPOAE measures (SmartOAE 4.31 USBez) and ABRs (SmartEP 3.62 USBez).

### 2.2. Subjects

The subject pool consisted of 55 mature rhesus monkeys (22 females, 33 males) between the ages of 15 and 28 years. The monkeys were born at the Wisconsin National Primate Research Center and entered the study when they were between the ages of 8 and 14 years. The monkeys were housed in similar controlled environments with individual weight-appropriate cages that al-

lowed visual and auditory contact with other monkeys and study personnel. Small environmental enrichment toys were provided. The environment was controlled at a temperature of approximately 20 °C, with a relative humidity of 50–65%. Twelve hours of lighting were provided between the hours of 6:00 AM and 6:00 PM. Further details on the basic study are presented in Kemnitz et al. (1993) and Ramsey et al. (2000).

The monkeys were entered into the project in three cohorts, and had participated for 162, 102, or 96 months (13.5, 8.5, and 8 years, respectively) at the time of testing. Monkeys were originally randomly assigned to one of two dietary groups; the control group was allowed to eat ad libitum and the experimental group was restricted to 70% of the calories of the control group, but supplemented such that vitamin and mineral content were equivalent between the groups. All monkeys had free access to water.

Monkeys were anesthetized with ketamine (15 mg/kg i.m.) and valium (0.75 mg/kg i.v.) for the auditory tests. Monkeys were included in the current study if there was no otoscopic or tympanometric evidence of ear canal occlusion or middle ear pathology. Two monkeys were eliminated on the basis of flat tympanograms, which suggested middle ear pathology unrelated to aging. Complete tympanometry data are presented elsewhere (Fowler et al., 2008).

### 2.3. Procedure for DPOAEs

The numbers of monkeys with complete DPOAE data are provided in Table 1 categorized by age, sex, and dietary condition. The participating monkeys included 22 female and 31 male monkeys ranging in age from 15–28 years. The controls had a mean age 20.5 years (range 15–26 years) and the caloric restricted monkeys had a mean age of 20.6 years (range 15–28 years).

DPOAEs were measured with a probe assembly inserted in the ear canal. For DPOAE measures, two primary frequencies ( $f_1$  and  $f_2$ , with  $f_2 > f_1$ ) were used with the  $f_2/f_1$  ratio fixed at 1.22. The levels of the primaries were held constant at  $L_1 = 65$  dB and  $L_2 = 55$  dB SPL. The distortion product of ( $2f_1 - f_2$ ) was measured for  $f_2$  frequencies 2211, 3125, 4416, 6250, and 8837 Hz. Sixty-four sweeps were averaged for each DPOAE.

### 2.4. Procedures for ABR thresholds

ABR thresholds were obtained for clicks and for 8, 16, and 32 kHz tone bursts. Five monkeys were retested two months after the all the monkeys were originally tested due to excess noise in the first recording. Forty-eight monkeys had reliable data for the three frequencies and 54 monkeys had reliable thresholds for clicks.

For the ABR, needle electrodes were placed at the brow ridge (positive input) and behind the right pinna (negative input) for channel 1 and from the brow ridge (positive input) and behind the left pinna (negative input) for channel 2. An electrode was placed at the shoulder for the ground. Electrode impedances were

**Table 1**  
Distribution of monkeys by dietary condition (CR = caloric restriction), sex, and months of participation in the study.

Diet condition	Sex	Months in the study			Total
		96	102	162	
CR	Female	7	4	–	11
	Male	7	–	10	17
Control	Female	9	2	–	11
	Male	6	–	8	14
Total	All	29	6	18	53

below 1 k ohm for all electrodes. Physiological filters were set to pass 100–3000 Hz.

Binaural stimuli were used to insure that the threshold of the better hearing ear was obtained. The stimuli were clicks and tone bursts at 8, 16, and 32 kHz with alternating polarity and a Blackman window with 2-ms rise-fall and 1 ms plateau times. Insert earphones (Etymotic ER-3A) were used to obtain the click and 8 kHz thresholds and high frequency transducers (IHS-3432) were used to obtain the 16 and 32 kHz thresholds. The high frequency transducers were placed 15 cm from the ear, with the center of the diaphragm level with the opening to the ear canal. Two trials of 700 sweeps were conducted at supra-threshold levels and 1000 sweeps near threshold. Signal levels began at 100 dB pSPL and descended in 20 dB steps at high levels and 10 dB steps near threshold including one or two steps below threshold. Threshold was defined as the lowest level for which a response was noted as present by an observer who had no identifying information regarding the status of the monkey.

2.5. Data analyses

For the DPOAE levels and ABR thresholds, data analyses were conducted using SAS 9.2 (2008) software in order to answer the questions concerning the effect of CR, sex, age and their interactions. Scatterplots were used to determine if age had a curvilinear relationship with any of the dependent variables. All two-way interactions were examined and removed if they were not significant at the  $p < .05$  level. Residuals from these models were examined and no extreme skewness was detected. To account for the paired data from each ear, SAS PROC MIXED was used to perform linear mixed models (LMMs) with DPOAE measures at the five frequencies as the dependent variables. For the ABR thresholds, data analyses were conducted using SAS PROC GLM with responses to clicks and the three tone pip frequencies as the dependent variables.

This study was approved by the Graduate School Institutional Animal Care and Use Committee (IACUC) of the University of Wisconsin-Madison.

3. Results

3.1. DPOAEs

Fig. 1 is a scatter plot of the individual DPOAE level data (ordinate) as a function of age in years (abscissa). Data were not

Table 2

Means and standard deviations (SD) for the DPOAE levels for the right and left ears for the five  $f_2$  frequencies tested, for the total monkeys tested and for the control and caloric restricted (CR) groups.

DPOAE $f_2$ frequency	Diet condition	Right ear		Left ear	
		Mean (dB SPL)	SD (dB SPL)	Mean (dB SPL)	SD (dB SPL)
2211 Hz	Total	9.1	8.5	9.2	7.9
	CR	6.6	9.2	8.5	8.5
	Control	12.0	6.7	9.9	7.3
3125 Hz	Total	3.8	8.0	5.0	7.6
	CR	2.9	8.8	4.5	8.3
	Control	4.9	7.1	5.6	6.9
4416 Hz	Total	-4.8	9.5	-3.7	10.4
	CR	-4.5	9.4	-3.8	10.0
	Control	-5.1	9.7	-3.5	10.1
6250 Hz	Total	3.1	9.6	2.3	11.0
	CR	1.6	9.7	1.0	11.9
	Control	4.8	9.2	3.8	9.9
8837 Hz	Total	8.2	9.4	7.7	9.4
	CR	8.3	9.5	8.4	10.5
	Control	8.1	9.4	6.9	8.2

significantly different for the two ears (paired  $t$ -test,  $t = -.247$ ,  $df = -264$ ,  $p = 0.8049$ ) and were significantly correlated (slope = 0.76,  $F = 352.7$ ,  $df = 1, 264$ ,  $p < .0001$ ). Data were averaged levels from both ears of each monkey for each of the five DPOAE  $f_2$  frequencies 2211, 3125, 4416, 6250, and 8837 Hz. Despite the variability of data from the individual monkeys, the regression lines through the data points for each frequency have essentially parallel slopes and all show the linear trend of decreasing level with increasing age. The slopes of DPOAE levels by age were  $-0.983$  for 2211 Hz,  $-0.928$  for 3125 Hz,  $-0.965$  for 4416,  $-0.807$  for 6250, and  $-0.881$  for 8837 Hz. All slopes were significantly different from 0 ( $p < 0.05$ ) except for the slope for the 6250 Hz DPOAE, which showed the same trend as the other frequencies. Overall, DPOAE levels declined just under 1 dB/year at each frequency.

Tables 2 and 3 show the DPAOE level data for the whole group of monkeys, with diet condition provided in Table 2 and sex differences provided in Table 3. Table 2 shows the means and standard deviations for the levels of the five DPOAE frequencies for the total sample and divided by caloric condition for both ears. Table 3 provides similar DPOAE level data, but in this table data are divided by sex. In both conditions, the DPOAE levels were not different between ears. Large standard deviations for all con-

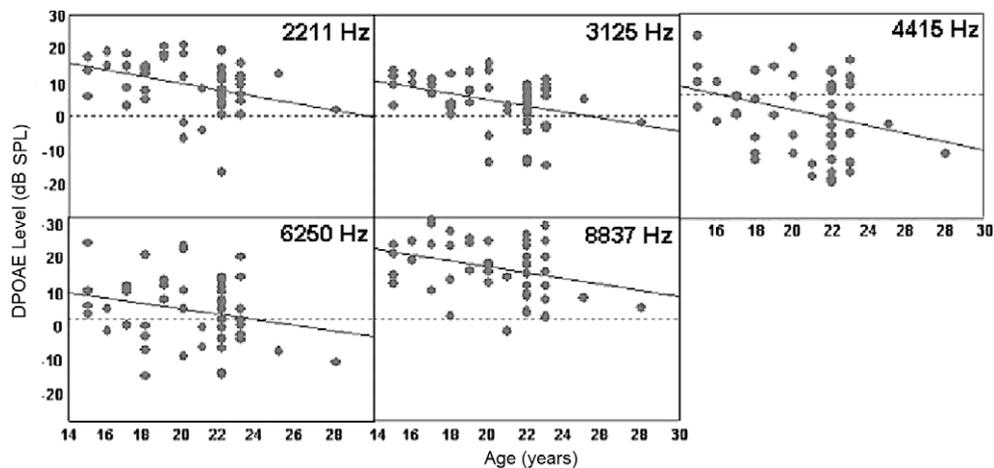


Fig. 1. Individual DPOAE levels (left and right ear data averaged) for all monkeys (ordinate) relative to age (years) at the five DPOAE  $f_2$  frequencies. The lines are the best fit regression lines through the data. Some data points are obscured by others.

**Table 3**

Means and standard deviations (SD) for the DPOAE levels for the male and female monkeys and the total monkeys for right and left ears and for the five  $f_2$  frequencies.

DPOAE $f_2$ frequency	Sex	Right ear		Left ear	
		Mean (dB SPL)	SD (dB SPL)	Mean (dB SPL)	SD (dB SPL)
2211 Hz	Total	9.1	8.5	9.2	7.9
	Female	9.0	10.0	9.9	9.0
	Male	9.2	7.4	8.6	7.1
3125 Hz	Total	3.8	8.0	5.0	7.6
	Female	3.3	9.6	3.7	8.0
	Male	4.2	6.8	5.9	7.4
4416 Hz	Total	-4.8	9.5	-3.7	10.4
	Female	-6.6	8.6	-3.1	8.8
	Male	-3.5	10.0	-4.1	11.5
6250 Hz	Total	3.1	9.6	2.3	11.0
	Female	2.4	8.7	0.9	11.2
	Male	3.6	10.3	3.4	10.8
8837 Hz	Total	8.2	9.4	7.7	9.4
	Female	7.1	7.2	8.9	8.2
	Male	9.1	10.7	6.8	10.3

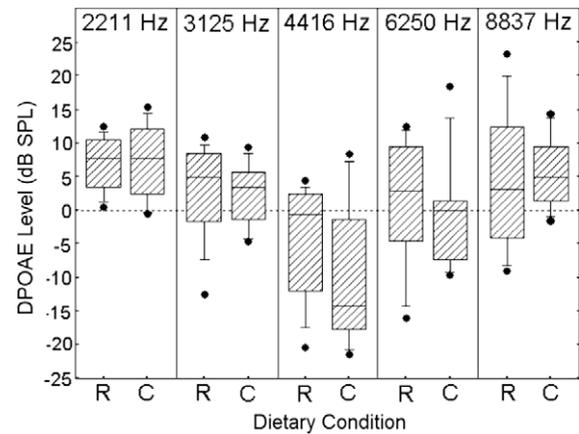
ditions reflect the variability across the wide age range of the monkeys tested.

For the DPOAE levels at the five frequencies, scatterplots indicated that trends for age were basically linear or null. Thus, in all subsequent LMM analyses, age was modeled as a linear covariate, and sex and diet as between-subjects factors. The all two-way interactions were tested, but none was statistically significant. The interactions were removed, and the LMM was used to analyze the main effects of diet and sex. Despite some apparent small differences in the data for the main effects, neither sex nor diet was significantly related to any of the DPOAE levels. Increasing age, however, was significantly related to decreases in levels for DPOAEs at 2211 Hz ( $p = 0.0055$ ), 3125 Hz ( $p = 0.0026$ ), and 4416 Hz ( $p = 0.0184$ ) when sex and diet were controlled. The relation of the DPOAE level at 6250 Hz with age was of marginal significance ( $p = 0.0554$ ) and the level at 8837 Hz did not vary with age after diet and sex were controlled.

The background noise levels, which ranged from -13 dB to -22 dB SPL across conditions (not shown), were evaluated to assure that there were no systematic differences among conditions that could have affected the DPOAE level measures. Noise levels for age, sex, diet, and their interactions were analyzed with the LMM and no significant effects were found. Noise levels, therefore, were ruled out as possible contributors to any differences noted in the DPOAE levels among variables.

The hypothesis that the monkeys were not in the study long enough for CR to have exerted a significant effect on the DPOAEs was tested with the cohort of monkeys that first entered the study. The monkeys were all males and had been in the study for 13.5 years at the time of testing. Fig. 2 shows box plots of the medians and ranges of DPOAE levels (in dB SPL) for the five frequencies for both caloric restricted (R) and control (C) monkeys in this cohort. The horizontal black bars within the boxes are the medians. Despite the large difference in the median for 4416 Hz, no significant differences were detected between the two groups, at least in part due to the variability among the individual monkeys.

The longest-participating cohort also showed an increased rate of loss in cochlear function at the highest frequencies compared to the younger cohorts. For this cohort of monkeys, the slopes of DPOAE level by age functions were -0.531 at 2211 Hz, -0.462 at 3125 Hz, -0.726 at 4416 Hz, -2.362 at 6250 Hz, and -2.054 at 8837 Hz. Overall, the monkeys with the longest participation are losing about 1/2 dB of DPOAE level in the lower frequencies and about 2 dB of DPOAE level in the higher frequencies each year. At



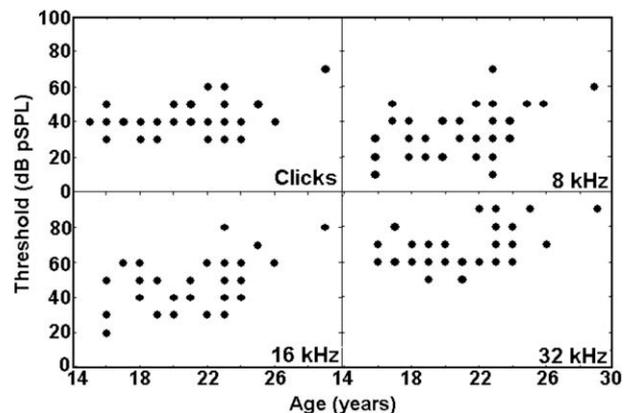
**Fig. 2.** Box plots of the median DPOAE levels (in dB SPL) for the five  $f_2$  frequencies for both caloric restricted (R) and control (C) monkeys in the cohort that was in the study for 13.5 years. The horizontal black bars within the boxes are the medians, the boxes enclose the 50 percentile range, the error bars delineate the 80th percentile, and black circles represent the maximum and minimum levels.

the highest frequencies, therefore, these older monkeys are beginning to experience a more rapid loss of cochlear function.

### 3.2. ABR thresholds

Scatterplots of the individual ABR thresholds for each of the four stimuli (clicks and 8, 16, and 32 kHz tone bursts) are provided in Fig. 3, which shows age on the abscissa and threshold in dB pSPL on the ordinate. Mean thresholds (with standard deviations) for clicks (Table 4) and tone bursts (Table 5) are given with the number of monkeys that had reliable thresholds. For each stimulus, the ABR thresholds increase with age.

There were no significant interaction effects for any of the ABR thresholds; thus, interaction effects were removed from subsequent models. For click thresholds, the effect of age was of marginal significance ( $p = 0.051$ ); however, there was a significant curvilinear effect ( $p = 0.0341$ ) indicating that responses start increasing after the age of 21 years. For responses at 8 kHz, the effect of age was statistically significant ( $p = 0.0295$ ) with thresholds increasing with age. The effect of diet was of marginal significance ( $p = 0.0543$ ) with controls having the higher thresholds. For responses at 16 kHz, the effect of age was of marginal significance ( $p = 0.068$ ); however, there was a significant curvilinear effect ( $p = 0.0485$ ), which indicated that thresholds start increasing after the age of 21 years. For responses at



**Fig. 3.** Individual ABR thresholds (in dB pSPL) for the individual monkeys by age (in years) for each of the signals (clicks and 8, 16, and 32 kHz). Some data points are obscured by others.

**Table 4**

Means (and SD) for the ABR thresholds to clicks (in dB pSPL) for male and female monkeys under caloric restriction (CR) and control (C) dietary conditions.

Diet condition	Sex	Number	Age (years)	Click threshold (dB pSPL)
CR	Male	17	22 (4)	41 (9)
CR	Female	10	21 (2)	39 (7)
C	Male	16	22 (3)	43 (11)
C	Female	11	20 (2)	38 (11)

32 kHz, the effect of age was significant ( $p = 0.0012$ ) with responses increasing with age. Sex was marginally significant ( $p = 0.0514$ ) with males having higher thresholds. The effect of time-in-study, after controlling for age, was not significant in any model.

#### 4. Discussion

The research questions in this study addressed the effects of CR, age, and sex on DPOAE level and ABR thresholds in aging monkeys. The strongest effect was that of age, with DPOAE levels decreasing linearly with age and ABR thresholds increasing with age. The only effect of diet was the marginally-significant lower thresholds at 8000 Hz for the monkeys on CR. The only effect of sex was marginally higher ABR thresholds for males at 32 kHz. Results are discussed with respect to the development of ARHL in the rhesus monkeys and their relation to studies of human auditory aging.

##### 4.1. Effect of age

The linear trend of decreasing DPOAE level with increasing age for each of the OAE frequencies in the current study is consistent with findings of other studies on aging rhesus monkeys. Direct comparisons, however, are limited because different DPOAE frequencies and measures were used in various studies. In the NIA study, Torre et al. (2004) reported that the DPOAE levels decreased significantly with age at all frequencies, with slopes indicating losses of approximately 0.7 dB/year for the frequencies 3000–5000 Hz and only 0.4 dB/year for 2000 Hz. The current study found that DPOAE level decreases approximately 0.95 dB per year for a similar range of frequencies (2211–4416 Hz). In the cohort participating for 13.5 years, however, the rate of decline doubled for the two highest DPOAE frequencies (6250 and 8817 Hz). These DPOAE findings were supported by the ABR thresholds for clicks and the 16 kHz tone bursts, which began increasing after the age of 21 years. A study of ARHL using behavioral thresholds in rhesus monkeys supports the current findings. Bennett and colleagues (Bennett et al., 1983) tested monkeys in three age groups (9, 24, and 31 years) with signals from 125 Hz to 32 kHz. The oldest monkeys had elevated thresholds for all frequencies. The difference in thresholds between monkeys aged 24 years compared to those aged 31 years was substantially larger than the difference between thresholds between monkeys aged 9 years compared to those aged 24 years.

The mid-frequency OAE levels in the current study dip below 0 dB SPL providing a “V” shaped function across frequencies. In the Torre and Fowler (2000) and Torre et al. (2004) studies, both

of which involved different groups of monkeys from the current monkeys and different test equipment, the DPOAE levels were flat across the frequencies tested. The “V” shaped function is not likely related to age. The lower DPOAE levels may be attributed to standing waves in the ear canal, calibration of the signal at the probe microphone, or differences in probes of the different instruments used (Mills et al., 2007). Behavioral thresholds for rhesus monkeys have shown 5–10 dB poorer thresholds at 4000 Hz relative to the surrounding frequencies (Behar et al., 1965; Pflugst et al., 1978), suggesting that differences between studies are not due entirely to the probes, but are more likely due to standing waves in the ear canals and plane of calibration.

Human studies of the effect of aging on DPOAE levels have also noted decreasing levels related to both stimulus frequency and age. Lonsbury-Martin et al. (1991) studied DPOAEs in aging using human participants aged 31–60 years, which is roughly equal to the ages of the monkeys in the current study considering the faster rate of aging in the monkeys. They reported age-related decreases in the DPOAE levels of humans that were on the order of 0.3 dB/year, which is roughly one third the rate of the level decrement in the monkeys. Dorn et al. (1998) tested humans ranging in age from 5 to 79 years, and noted decreasing levels as a function of age, with increasing rates of decline at  $f_2$  frequencies of 6000 and 8000 Hz, the same two frequencies noted in the current study for the older monkeys. Both the Lonsbury-Martin et al. and Dorn et al. studies attempted to reduce variability from non-aging factors in the human data by controlling pure tone thresholds of the participants. Whereas this approach was not possible in the monkeys, the monkeys had fewer risk factors for hearing loss given their controlled environments and diets.

The ABR thresholds also decline with age for all the signals used in the current study. Click thresholds were compared between the monkeys reported here and compared to their thresholds obtained 5 years earlier (Fowler et al., 2002). The individual data are given in Fig. 4, with the earlier thresholds on the abscissa and the current thresholds on the ordinate, and the best fit regression line through the data. The methods for obtaining these thresholds were slightly different in that the previous thresholds were obtained with unilateral stimuli and in 5 dB steps, whereas the current thresholds were obtained with binaural stimuli and in 10 dB steps. The average for the current thresholds was 5 dB lower than the average for the previously obtained thresholds, perhaps reflecting these methodological changes. The thresholds obtained in the two studies were significantly correlated ( $r = 0.523$ ,  $p < 0.0001$ ) and can be interpreted as showing essentially no progression overall in thresholds in the past 5 years for the surviving monkeys.

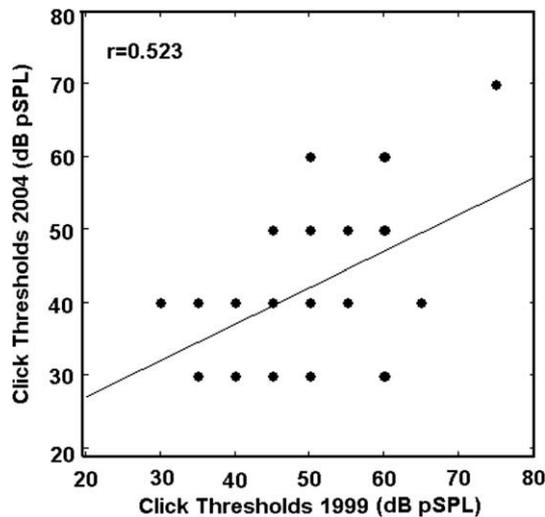
##### 4.2. Effect of CR

There were no significant effects of diet condition on cochlear function as measured with DPOAEs elicited by supra-threshold signals. The only other study of CR on auditory function in rhesus monkeys is Torre et al. (2004), which reported on 50 male rhesus monkeys aged 13–36 years in the NIA study. They found no significant differences in DPOAE levels related to caloric condition at  $f_2$  frequencies from 2000 to 5000 Hz. Together, these two studies

**Table 5**

Means (and standard deviations) for the ABR thresholds (in dB pSPL) to tone bursts (8, 16, 32 kHz) for the male and female monkeys under caloric restricted (CR) and control (C) dietary conditions.

Diet condition	Sex	Number	Age (years)	8 kHz (dB pSPL)	16 kHz (dB pSPL)	32 kHz (dB pSPL)
CR	Male	14	22 (3)	36 (14)	54 (15)	68 (14)
CR	Female	11	21 (2)	30 (12)	43 (10)	66 (14)
C	Male	14	22 (3)	39 (15)	50 (15)	72 (8)
C	Female	9	20 (2)	33 (8)	43 (11)	64 (5)



**Fig. 4.** Individual click-evoked ABR thresholds for the surviving monkeys with thresholds from 1999 (abscissa) compared to thresholds from 2004 (ordinate). The line is the best fit regression line for the data. Some data points are obscured behind others.

included a total of 103 monkeys and were consistent in failing to find significant effects from caloric condition on DPOAE levels for a range of signals between 2000 and 8817 Hz.

ABR thresholds were obtained in these two studies as well. Both Torre et al. (2004) and the current study failed to note any effects of caloric restriction on click-elicited responses. The current study, however, included ABR thresholds elicited with high frequency tone bursts, and found marginal significance at 8000 Hz, with the CR monkeys having lower thresholds. These findings suggest that clicks may not be effective stimuli for revealing subtle threshold differences that are emerging related to diet condition.

Torre et al. (2004) hypothesized that male monkeys may be resistant to the effects of CR or may require longer durations of CR in order to exhibit any protective effects on auditory function. The current study supports the interim conclusion that preservation of auditory function is not evident in male monkeys in the first 13.5 years of the diet, at least as measured by supra-threshold DPOAEs and click-elicited ABR thresholds. The finding of marginal significance for the 8000 Hz thresholds, however, suggests that more sensitive test measures may be needed. Future studies should concentrate on ABR thresholds to tonal stimuli at a wider range of frequencies. Thresholds for DPOAEs also may prove more sensitive than measures at supra-threshold levels (Lasky et al., 1999).

One variable in the Torre et al. (2004) study and the current study of CR in rhesus monkeys is the age at which they began restricted diets. In the current study all monkeys ate normal diets through their growth and development and began the diets only after they had reached young adulthood. Monkeys in the NIA study began CR diets at various stages in their development (Lane et al., 1992). Thus far in the two experiments, the age at which the monkeys began the CR has yielded no apparent differences on the development of ARHL.

Duration of CR may also be a significant factor in affecting the development of ARHL. Rhesus monkeys are the animals with the longest lifespan yet to be tested with CR, so the duration of CR necessary to affect the development of ARHL is not known. Support for the requirement of longer durations of CR is provided by a study of sarcopenia in the same cohort of older monkeys in the Wisconsin study. In that study, a duration of CR over 14 years was required to show the benefits on age-related loss of muscle mass (Coleman et al., 2008). A study of CBA/J mice indicated that CR begun in early

life or midlife exhibited less presbycusis in later life than ad libitum fed mice, but only when the CR was maintained until the time of testing (Sweet et al., 1988). At this point, the oldest cohort of monkeys is showing increasing rates of hearing loss. If the monkeys follow the pattern of the mice, CR may finally affect cochlear function over the next decade as the hearing losses accelerate and more monkeys enter old age.

The effect of genetic regulation of the response to CR has been noted in several species. Evidence in mice suggests that genes specific to individual strains play a significant role in how CR affects the auditory system (Willott et al., 1995). The rhesus monkeys are fairly heterogeneous genetically as are humans, and the genes necessary for benefiting from CR have not yet been identified in the monkeys or in humans.

Finally, presbycusis results in the degeneration of the cochlear hair cells as well as neural elements of the auditory system in humans (Schuknecht and Gacek, 1993; Frisina and Walton, 2006) and in mice (Park et al., 2000; Someya et al., 2007), suggesting that threshold measures may be too simple a task for the neural system to reveal subtle losses. Further monitoring may be necessary to see possible protective effects from CR as the ARHL progresses.

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