The Relationship Between Metabolic Presbycusis and Serum Paraoxonase/Arylesterase Activity

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Received: 9 May 2011 / Accepted: 15 November 2011 / Published online: 20 December 2011
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Abstract To determine the presence of a relationship between metabolic presbycusis and serum paraoxonase/arylesterase activity. A total of 30 patients who had been admitted to the Ear, Nose, and Throat (ENT) Clinic of Firat University Medical Faculty and diagnosed as metabolic presbycusis were included in the study. The control group was composed of 30 healthy volunteers. Pure tone audiometry and impedencemeter were performed on all subjects included in the study at the audiometry laboratory of the ENT clinic. The presence of a regular hearing curve, a symmetrical sensorineural hearing loss more than 25 dB with preserved speech discrimination were accepted as criteria for metabolic presbycusis. Blood samples were drawn from the patients prior to the hearing tests. The sera were separated for measurements of total cholesterol, triglyceride, high-density lipoprotein, very low-density lipoprotein, low-density lipoprotein, human serum paraoxonase and arylesterase levels, respectively. No statistically significant difference was found between the patient and the control groups in terms of age and gender. Paraoxonase, arylesterase and paraoxonase/arylesterase, high-density lipoprotein levels were found to decrease in the study group and the difference was found to be statistically

significant compared to the control group ($P < 0.001$). Low-density lipoprotein and total cholesterol levels were found to increase in the patient group and the difference was found to be significant compared to the control group ($P < 0.001$). This study puts forth that especially the type of nutrition and life style are very important with regard to metabolic presbycusis. Furthermore, the results of this study make us think that there could be a relationship between metabolic presbycusis and cardiovascular diseases. In this case, metabolic presbycusis may be a determining parameter in the early diagnosis of cardiovascular diseases. We consider that this study may be the pioneer for further studies conducted with larger patient numbers.

Keywords Metabolic presbycusis · Paraoxonase · Arylesterase · Lipid

Introduction

Presbycusis that may be defined as aging of the hearing mechanism has been classified as sensorial, neural, metabolic, mechanical presbycusis by Schuknecht [1]. In the geriatric population, changes are observed in the structure of middle ear. However, this anatomical alteration does not change the resonance frequency of middle ear; hence, sound conduction is not blocked. As metabolic presbycusis arises from a defect and vascular changes in physical and chemical processes providing energy production in the cochlea, hearing loss develops as the result of atrophy of stria vascularis. The stria vascularis regulates the chemical and bioelectrical balance and metabolic health of the cochlea. This atrophy leads to a hearing loss in which a regular hearing curve is observed on the audiogram. This is because the cochlea is affected

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completely. The scores of speech discrimination are preserved. Presbycusis tends to be seen in subjects between 30 and 60 years of age. It progresses slowly and may be familial [2].

Paraoxonase (PON) is the active metabolite of parathion, an organic phosphorus insecticide synthesized in the liver, and having both arylesterase and PON (aryldialkyl phosphatase; E.C.3.1.8.1) activity. It is a serum esterase that has the ability of hydrolyze paraoxon [3, 4]. Human serum paraoxonase (PON1) is a 354-aminoacid protein with a molecular weight of 43 kDa, and associated with high-density lipoprotein (HDL). PON1 is located in the outer surface of cell membrane, which passes onto HDL via lipoproteins. PON1 is also effective on hydrogen peroxide along with lipid peroxides. Thus, it is also considered to have peroxidase-like activity [5, 6]. It also provides protection against bacterial endotoxins via lipopolysaccharide inactivation. It has also been reported to show antioxidant and anti-inflammatory properties [7, 8].

Human serum paraoxonase and arylesterase (ARE) are enzymes of the esterase group that are encoded by the same gene and have similar active centers [9]. Although PON1 is known to display polymorphic alteration, the ARE enzyme does not show a genetic polymorphic alteration. The PON1 enzyme also has antioxidant function because of its neutralizing capacity.

Human serum paraoxonase has been shown to possess a protective effect in the atherosclerotic process by contributing to the protective effect of HDL from atherosclerosis and by preventing lipoprotein peroxidation and LDL-cholesterol oxidation. The PON1 level has been shown to decrease in cardiovascular diseases, smokers, hypercholesterolemia, advanced age, obesity, menopause and renal insufficiency [6, 7, 10, 11].

Antioxidants are a group of chemical substances produced by the cells of the body and obtained from foods. These chemicals protect the cells and the immune system against free radicals [12]. A free radical is a molecule that has one or more unmapped electrons. The harmful effects of free radicals are among the most important causes of early aging [13]. The unsaturated bonds of membrane cholesterol and fatty acids easily react with free radicals and lipid peroxides are formed. Lipid peroxidation may be autocatalytic and spread along all the membrane. The cell may die unless this is prevented [14]. The antioxidant protection systems weaken with an increase of free radicals appearing with advanced age. The self repair property of the cells decreases [14].

Hyperlipoproteinemia is known to be an important etiological factor in presbycusis and progressive sensorineural hearing loss [15]. Antioxidant levels may be suggested to be affected in metabolic presbycusis and perhaps

presbycusis may be treated successfully with antioxidant treatment.

In this study, the rates of PON1, ARE, and PAN/ARE and the levels of HDL, LDL, very low-density lipoprotein (VLDL), triglyceride (TG) and total cholesterol (TC) were assessed and compared with a control group composed of subjects with intact hearing of the same age group, and the etiology of presbycusis was attempted to be explained.

Materials and Methods

A total of 30 patients who had been admitted to Firat University Ear, Nose and Throat Clinic and diagnosed as metabolic presbycusis were included in the study. The control group was composed of 30 healthy volunteers. Written approval was obtained from the Firat University Ethics Committee of Human Investigations prior to the study. Verbal informed consents of the subjects in the patient and the control groups were obtained after having been informed. All individuals included in the study underwent hearing tests with pure tone audiometry, acoustic reflex thresholds with impedencemeter and tympanograms at the audiology laboratory of Ear, Nose and Throat Department. The audiometric tests were performed in the standard acoustic control rooms using the inter-acoustic AC-40 audiometry device calibrated according to ISO 164 standards. Audiometric tests were performed after the required measurements had been made in order to avoid temporary threshold elevation. Air way was assessed in a frequency range of 250–6,000 Hz and bone conduction was tested between 500 and 4,000 Hz. Values below 25 dB were accepted as the limit for normal hearing threshold according to the ISO-1964 standard. Individuals who had pathologies that could lead to secondary lipid disorders, cardiovascular diseases, diabetes mellitus, renal insufficiency, history of ototoxic drug use, ear disease and head and ear trauma were excluded from the study. The presence of a regular hearing curve, and a symmetrical sensorineural hearing loss of more than 25 dB with preserved speech discrimination were accepted as criteria for metabolic presbycusis.

Obtaining Blood Samples

Venous blood samples were obtained from the patients before the hearing tests. The sera were separated for measurement of TC, TG, HDL, VLDL, LDL, PON1, and ARE levels, respectively. The sera were transferred into unused cover tubes. The tubes were stored at -20°C in the deep-freezer and studied as groups. TC, TG, HDL, and LDL levels were studied in the Olympus AU 600 autoanalyzer (Olympus Optical Co., Japan) using Randox kits.

Paraoxonase (PON), ARE Analysis

The same sera pool was used at the beginning of the study and it was tried to study the fresh sera as much as possible. The sera pool was stored at -20°C in the deep-freezer for studies planned to be performed the next day. Blood samples obtained from the same group were used for testing of hemolysis and the plasma samples with EDTA.

Paraoxon was used as the substrate for the measurement of PON enzyme activity and phenylacetate was used as the substrate for the measurement of ARE. All chemicals used were in analytic purity and obtained from Merck and Sigma. In the measurement of PON activity in the serum pool, the basal PON activities were analyzed by making measurements using glycine and Tris–HCl buffers containing CaCl_2 in different concentrations and paraoxon (*O,O*-diethyl-*O-p*-nitrophenylphosphate; Sigma Co, London, UK) in different concentrations at different pH and temperatures (37 and 25°C). NaCl-stimulated PON activities were detected by adding 0.5 and 1 mol/l NaCl into the same solutions. For the measurement of ARE activity, phenylacetate was added to Tris–HCl and glycine buffers as the substrate instead of paraoxon, so that the last concentration would be 1 and 2 mmol/l. 4-Nitrophenol formed as the result of enzymatic hydrolysis of PON1 and phenol formed as the result of enzymatic hydrolysis of ARE were measured in the Techcomp 8500 II UV/Vis spectrophotometer. Additionally, wave-length screening of the products formed as the result of the reaction was performed in the Techcomp 8500 II UV/Vis spectrophotometer. The product formed following the enzymatic reaction when studying the PON1 activity showed maximum absorbance at 405 and 412 nm wavelengths; however, the measurements were preferred to be made at this wavelength as a more ideal peak length was obtained at 412 nm. In the measurement of ARE enzyme activity, the maximum peaks were obtained at 270 and 278 wavelengths and the measurements were decided to be made at 270 nm. For PON activity, 1 unit was described as 1 nmol 4-nitrophenol/ml serum/min; for ARE activity, 1 unit was defined as 1 μmol phenol/ml serum/min [16, 17].

Statistical analyses were performed using the SPSS for Windows version 11.0 package program. The independent Student's *t* test was used for comparisons of the patient and the control groups. A *P* value of <0.05 was accepted to be statistically significant.

Results

The patient group included 13 females and 17 males; the control group included 15 females and 15 males. The mean age of the patient group was 54 ± 5.5 years (range 32–60) and the mean age of the control group was

53.2 ± 9.6 years (range 31–60). No statistically significant difference was found between the groups in terms of age and gender. Comparison of PON 1, ARE, PON/ARE, TC, HDL, LDL, VLDL, and TG values of the patient and control groups has been presented in Table 1. The mean frequency-specific hearing thresholds of the patient and control groups have been displayed in Table 2.

Discussion

Presbycusis is a progressive, bilateral, symmetrical, sensorineural hearing loss appearing in old age.

Table 1 Comparison of PON 1, ARE, PON/ARE, TC, HDL, LDL, VLDL and TG values of the patient and control groups

Parameter	Patient group (<i>n</i> = 30)	Control group (<i>n</i> = 30)	<i>P</i> value
PON	204.4 ± 9.9	323.5 ± 82.4	0.001*
ARE	216.6 ± 36.3	240.8 ± 53.1	0.044*
PON/ARE	0.9 ± 0.1	1.3 ± 0.2	0.001*
TC	262 ± 45.4	199.2 ± 42.0	0.001*
HDL	25.1 ± 4.2	35.0 ± 7.3	0.001*
LDL	181.6 ± 38.1	138.2 ± 40.8	0.001*
VLDL	45.4 ± 16.6	46.7 ± 23.1	0.810
TG	231.2 ± 75.7	233.7 ± 61.1	0.920

PON paraoxonase, ARE aryl esterase, TK total cholesterol, HDL high-density lipoprotein, LDL low-density lipoprotein, VLDL very-low-density lipoprotein, TG triglyceride

* Statistically significant ($P < 0.05$)

Table 2 Comparison of the mean hearing thresholds of the patient and control groups

Frequency (Hz)	Mean hearing threshold (dB HL)		<i>P</i> value
	Patient group (<i>n</i> = 30)	Control group (<i>n</i> = 30)	
250 R	54.16 ± 9.00	18.16 ± 9.14	0.0001*
250 L	55.16 ± 11.00	18.00 ± 9.43	0.0001*
500 R	51.50 ± 9.28	14.33 ± 7.15	0.0001*
500 L	53.50 ± 10.59	13.16 ± 6.62	0.0001*
1,000 R	52.00 ± 9.15	12.66 ± 6.66	0.0001*
1,000 L	53.66 ± 11.51	13.66 ± 6.14	0.0001*
2,000 R	56.16 ± 7.27	16.33 ± 6.81	0.0001*
2,000 L	60.16 ± 10.21	18.00 ± 7.02	0.0001*
4,000 R	60.00 ± 8.30	21.33 ± 8.29	0.0001*
4,000 L	60.16 ± 10.21	23.50 ± 9.01	0.0001*
6,000 R	64.50 ± 7.11	26.66 ± 8.64	0.0001*
6,000 L	64.00 ± 7.58	27.50 ± 9.71	0.0001*

R right, L left

* Statistically significant ($P < 0.005$)

Comprehensive investigations have been performed on the pathological changes in the inner ear in presbycusis. However, the exact mechanism in the development of presbycusis is still unknown [18, 19]. Histological changes may develop in the auditory system, hairy cells of the cochlea, and the hearing cortex in the temporal lobe due to aging. The anatomical level at which these changes are seen is correlated with the clinical findings and hearing tests indicating the severity of these changes [1]. Schuknecht and Gacek [2] researched the histological changes in the cochleas of humans with presbycusis and described four regions related to cochlear aging. Presbycusis was divided into four types according to these regions. *Sensorial presbycusis* is defined as the condition caused by epithelial atrophy along with loss of hairy cells and stromal cells in the Corti's organ. This condition progresses towards the apex beginning from the cochlear base. Progression is slow. These changes begin after middle age and accompany a sharp decrease in high frequency value on the audiogram. *Neural presbycusis* has been suggested to be caused by atrophy of neural cells in the cochlea and the central neural pathways. A sharp decrease in high frequency is not observed on the audiogram as the atrophy develops along with all the cochlea. There is a severe decrease in speech discrimination scores. This decrease may be seen before the hearing loss. As *metabolic presbycusis* arises from a defect in, and vascular changes in the physical and chemical functions providing energy production in the cochlea, hearing loss develops as a result of atrophy of the stria vascularis. The stria vascularis regulates the chemical, bioelectrical balance and metabolic health of the cochlea. This atrophy leads to a hearing loss causing a regular hearing curve to be observed in the audiogram. This occurs due to the fact that the cochlea is completely affected. Speech discrimination scores are preserved. It tends to be seen in individuals between 30 and 60 years of age. It progresses slowly and may be familial. *Mechanical presbycusis* is caused by thickening and secondary stiffening of the cochlea basilar membrane. Thickening is more severe in the basal part of the cochlea in which the basilar membrane is narrow. This is correlated with slowly progressing high frequency hearing loss. The score of speech discrimination is moderate in this type of presbycusis. In this study, we included patients with presbycusis in whom a regular hearing curve was observed, scores of speech discrimination were preserved and with an age range of 30 years as the minimum and 60 years as the maximum. We did not include all the presbycusis patients in this study, because we knew that the etiology of presbycusis was related to many factors. Publications indicating that a relationship could be present between hearing loss and lipid levels encouraged us for this study conducted with patients with metabolic presbycusis.

Hyperlipoproteinemia has been reported to cause anoxia in the sensitive cells of the inner ear and possible development of sudden fluctuating or progressive hearing and vestibular disorders related to this [15, 20]. Saito et al. [15] found that a high fat containing diet caused a 40% increase in the hearing threshold in their study carried out with guinea pigs. They observed vacuolar degeneration and parenchymal changes developing in the inner ear, stria vascularis and Corti's organ of guinea pigs. Spencer et al. [21] found definite hyperlipoproteinemia in 42.3% and borderline hyperlipoproteinemia in 8.66% of 300 patients who had inner ear disease with cochlear and/or vestibular symptoms. Kojima et al. [22] stated that hearing loss improved following treatment of hyperlipidemia in patients with chronic phase sudden hearing loss. Hyperlipoproteinemia has been reported to be seen together with sudden fluctuating or progressive vestibular and hearing disorders, and hyperlipoproteinemia could probably cause anoxia in the sensitive cells of the inner ear via vascular blockade.

Paraoxonase has been reported to protect LDL from oxidation by hydrolyzing lipid peroxides, to be effective on hydrogen peroxide (peroxidase-like activity), to have the property of metabolizing fat-soluble radicals and moreover, to show antioxidant and anti-inflammatory properties [5, 6]. In fact, PON activity has been reported to be low in conditions in which oxidative stress is increased such as diabetes mellitus, hypercholesterolemia and cardiovascular diseases [5, 6, 23]. We did not encounter a study in the literature investigating the lipid levels in patients with metabolic presbycusis, the relationship between PON1 and ARE levels and lipid levels, and the hearing levels. In this study, we found a statistically significant difference between the patient and the control groups in terms of PON1, ARE, HDL, LDL, TC, TG values.

Serum PON1 levels are affected by diet, acute phase proteins, pregnancy, and disorders affecting the apoA1 metabolism [24]. PON1 hydrolyze organophosphates like PON, aromatic esters like phenylacetate and lipid peroxidation products, and decreases their accumulation. With this property, PON1 prevents the acceleration of atherosclerosis and gains anti-atherogenic property [3, 4]. PON1-a part of HDL cholesterol is a protective enzyme in the atherosclerotic process via contributing to the preventive effect of HDL on atherosclerosis and preventing lipoprotein peroxidation and LDL-cholesterol oxidation [3, 4]. The results of this study made us think that there could be a relationship between metabolic presbycusis and cardiovascular diseases. Perhaps, metabolic presbycusis may be used as a parameter in the early diagnosis of cardiovascular diseases.

There are many studies indicating that free radicals lead to neuron destruction and atherosclerotic changes [25–27]. Defects in the antioxidant system counteracting the free radicals which are the cause of oxidative damage, are the cause of aging [26, 27]. Decreased perfusion of the cochlea

and attenuation in the antioxidant system cause accumulation of reactive oxygen metabolites and this causes a damage in mitochondrial DNA in the neural structures of the inner ear. Damage of mitochondrial DNA decreases the oxidative phosphorylation and thus, the neural functions of the inner ear diminish. In the study of Dai, he found that damaged mitochondrial DNA caused alterations in the anatomical structure of the inner ear [28].

While oxidative damage causes aging, the fact that antioxidants prolong life has also been proven experimentally [27]. Antioxidant enzymes were analyzed in patients with presbycusis and a relation was detected between the antioxidant enzyme genes and appearance of presbycusis [18]. In experimental models, hearing levels were evaluated in antioxidant therapy given as combination in hearing loss emerging with age, and improvement in hearing levels was proven with the BERA test [29]. In tests performed on humans following experimental studies, age-related hearing loss was shown to be prevented with diet and antioxidants [30]. In our study, PON1 and ARE values were found to be lower in metabolic presbycusis patients, compared to the control group. This was statistically significant. This condition made us think that PON1 and ARE could be the cause of metabolic presbycusis as a result of the loss of antioxidant properties and contribution to the atherosclerotic process.

Increase in blood cholesterol levels is effective on aging, causing an increase in the free radical level. One of the enzymes that has antioxidative and anti-inflammatory features is PON1. PON1 is associated with HDL. HDL also has protective functions different from other cholesterol [31]. The protective effect of HDL against LDL-C oxidation has been shown to be higher than antioxidant vitamins [3]. In our study, PON1, ARE, and HDL were found to be lower, and TC and LDL levels were found to be higher in the patient group, compared to the control group. This was statistically significant.

Conclusion

In our study, PON1, ARE and the PON/ARE ratio and HDL levels were found to be lower in the metabolic presbycusis group compared to the control group. The LDL/TC ratio was found to be statistically significantly high. This condition puts forth that especially diet and life style are very important with regard to presbycusis. Furthermore, a relationship could be suggested to exist between metabolic presbycusis and cardiovascular diseases. Perhaps this condition could be used as a determinant parameter in the early diagnosis of cardiovascular diseases. In the light of these data, we consider that this study could be the pioneer for further studies on this subject.

References

- Schuknecht HF (1964) Further observations on the pathology of presbycusis. *Arch Otolaryngol* 80:369–382
- Schuknecht HF, Gacek MR (1993) Cochlear pathology in presbycusis. *Ann Otol Rhinol Laryngol* 102:1–16
- Juretic D, Tadijanovic M, Rekec B, Simean-Rudolf V, Reiner E, Baricic M (2001) Serum paraoxonase activities in hemodialyzed uremic patients: cohort study. *Clin Sci* 42:146–150
- Li WF, Costa LG, Furlong CE (1993) Serum paraoxonase status: a major factor in determining resistance to organophosphates. *J Toxicol Environ Health* 40:337–346
- Durrington PN, Mackness B, Mackness MI (2001) Paraoxonase and atherosclerosis. *Arterioscler Thromb Vasc Biol* 21:473–480
- Mackness MI, Mackness B, Durrington PN (2002) Paraoxonase and coronary heart disease. *Atheroscler Suppl* 3:49–55
- Ferretti G, Bacchetti T, Busni D, Rabini RA, Curatola G (2004) Protective effect of paraoxonase activity in high-density lipoproteins against erythrocyte membranes peroxidation: a comparison between healthy subjects and type1 diabetic patients. *J Clin Endocrinol Metab* 89:2957–2962
- Ferretti G, Bacchetti T, Moroni C et al (2005) Paraoxonase activity in high-density lipoproteins: a comparison between healthy and obese females. *J Clin Endocrinol Metab* 90:1728–1733
- Eckerson HW, Wyte CM, La Du BN (1983) The human serum paraoxonase/arylesterase polymorphism. *Am J Hum Genet* 35:1126–1138
- Mackness B, Durrington PN, Mackness MI (1998) Human serum paraoxonase. *Gen Pharmacol* 31:329–336
- Aviram M (1999) Does paraoxonase play a role in susceptibility to cardiovascular disease? *Mol Med Today* 5:381–386
- Balsano C, Alisi A (2009) Antioxidant effects of natural bioactive compounds. *Curr Pharm Des* 15:3063–3073
- Valko M, Leibfritz D, Moncol J, Cronin MT, Mazur M, Telser J (2007) Free radicals and antioxidants in normal physiological functions and human disease. *Int J Biochem Cell Biol* 39:44–84
- Beckman KB, Ames BN (1998) The free radical theory of aging matures. *Physiol Rev* 78:547–581
- Saito T, Sato K, Saito H (1986) An experimental study of auditory dysfunction associated with hyperlipoproteinemia. *Arch Otorhinolaryngol* 243:242–245
- Eckerson HW, Romson J, Wyte CM, La Du BN (1983) Human serum paraoxonase polymorphism: identification of phenotypes by their responses to salts. *Am J Hum Genet* 35:214–227
- Mueller RF, Hosnung S, Furlong CE, Anderson J, Giblett ER, Motulsky AG (1983) Plasma paraoxonase polymorphism. A new enzyme assay, population, family, biochemical and linkage studies. *Am J Hum Genet* 35:393–408
- Bared A, Ouyang X, Angeli S, Du LL, Hoang K, Yan D, Liu XZ (2010) Antioxidant enzymes, presbycusis, and ethnic variability. *Otolaryngol Head Neck Surg* 143:263–268
- Huang Q, Tang J (2010) Age-related hearing loss or presbycusis. *Eur Arch Otorhinolaryngol* 267:1179–1191
- Michikawa T, Nishiwaki Y, Kikuchi Y, Hosoda K, Mizutani K, Saito H, Asakura K, Milojevic A, Iwasawa S, Nakano M, Takebayashi T (2009) Serum levels of retinol and other antioxidants for hearing impairment among Japanese older adults. *J Gerontol A Biol Sci Med Sci* 64:910–915
- Spencer J (1973) Hyperlipoproteinemias in the etiology of inner disease. *Laryngoscope* 3:639–678
- Kojima Y, Ito S, Furuya N (2001) Hearing improvement after therapy for hyperlipidemia in patients with chronic-phase sudden deafness. *Ann Otol Rhinol Laryngol* 110:105–108
- Mackness MI, Harty D, Bhatnagar D, Winocour PH, Arrol S, Ishola M, Durrington PN (1991) Serum paraoxonase activity in

- familial hypercholesterolaemia and insulin-dependent diabetes mellitus. *Atherosclerosis* 86:193–199
24. Sutherland WHF, Walker RJ, Jong SA, Van Rij AM (1999) Reduced postprandial serum paraoxonase activity after a meal rich in used cooking fat. *Arterioscler Thromb Vasc Biol* 19:1340–1347
 25. Greenstock CL (1993) Radiation and aging: free radical damage, biological response and possible antioxidant intervention. *Med Hypotheses* 41:473–482
 26. Nohl H (1993) Involvement of free radicals in aging: a consequence or cause of senescence. *Br Med Bull* 49:653–667
 27. Harman D (1993) Free radical involvement in aging. *Pathophysiology and therapeutic implication*. *Drugs Aging* 3:60–80
 28. Dai P, Yang W, Jiang S (2004) Correlation of cochlear blood supply with mitochondrial DNA common deletion in presbycusis. *Acta Otolaryngol* 124:130–136
 29. Heman-Ackah SE, Juhn SK, Huang TC, Wiedmann TSA (2010) Combination antioxidant therapy prevents age-related hearing loss in C57BL/6 mice. *Otolaryngol Head Neck Surg* 143:429–434
 30. Bielefeld EC, Tanaka C, Chen GD, Henderson D (2010) Age-related hearing loss: is it a preventable condition? *Hear Res* 264:98–107
 31. Pietinen P, Hartman AM, Haapa E, Rasanen L, Haapakoski J, Palmgren J, Albanes D, Virtamo J, Huttunen JK (1988) Reproducibility and validity of dietary assessment instruments. I. A self-administered food use questionnaire with a portion size picture booklet. *Am J Epidemiol* 128:655–666