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Coenzyme Q10 and rikkunshito prevent age-related changes in mouse otolith morphology and function

Keita Ueda [a,*], Takao Imai [a], Tadao Okayasu [a], Tatsuhide Tanaka [b], Kouko Tatsumi [b], Akio Wanaka [b], Tadashi Kitahara [a]

[a] Department of Otolaryngology-Head and Neck Surgery, Nara Medical University, Japan
[b] Department of Anatomy and Neuroscience, Faculty of Medicine, Nara Medical University, Japan

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ABSTRACT

Otoliths play an important role in maintaining body balance, and age-related decline in theis function and morphological integrity can lead to falls. In recent years, the herbal medicine rikkunshito (RKT) and the anti-oxidant coenzyme Q10 (CoQ10) have been studied for their anti-aging properties; however, their effects on otoliths remain unknown. Therefore, we aimed to investigate whether RKT and CoQ10 can prevent age-related functional and morphological changes in otoliths. To this end, 30 male and 30 female 8-week-old C57BL6N mice were used in this study. The mice were divided into three groups: a control group, CoQ10 group (0.2 % CoQ10 special diet), and RKT group (3 % RKT special diet). At 80 weeks of age, micro-computed tomography (μCT) images were taken and analyzed for otolith volume and CT number. Furthermore, eye movements induced by the linear vestibulo-ocular reflex (LVOR) were analyzed to assess otolith function.

Results: revealed that the RKT group had a significantly smaller volume of the 3 dimensional utriclar CT model (male mice; $p = 0.0281$, Steel test) and a significantly higher utricular CT number (male mice; $p = 0.0104$, Dunnett test) than the control group. The RKT group had a significantly weaker LVOR (male mice; lateral 1.3G stimulation; $p = 0.00681$, Dunnett test) (male mice; longitudinal 1.3G stimulation; $p = 0.0183$, Dunnett test) (male mice; longitudinal 0.7G stimulation; $p = 0.00322$, Dunnett test) than the control group. The CoQ10 group exhibited a significantly stronger utricle-induced LVOR than the control group (female mice; lateral 0.7G stimulation; $p = 0.0133$, Steel test).

In conclusion, RKT prevented age-related utricular morphological changes, but did not prevent age-related otolith functional changes in male mice. CoQ10 prevented age-related utricular functional changes for low frequency stimulation in female mice.

1. Introduction

Age-related decline in postural stability is associated with the deterioration of all factors contributing to body balance [1]. In the United States, balance disorders affect 75 % of individuals aged 70 and older [2]. Declining vestibular, visual, and somatosensory function compromises balance, leading to falls [3]. Vestibular dysfunction has been associated with an eight-fold increased risk of falling, raising concerns about fall-related morbidity and mortality [4,5]. Age-related vestibular decline has been shown to correlate with reduction in the number of vestibular hair cells (HCs) and neurons [1]. “Presbyvestibulopathy" and "Vestibular frailty" were recently proposed by Barany Society [6] and Nara Medical University [7] respectively as a broad term describing age-related vestibular disorders, intended to encompass mild or incomplete vestibular loss due to the normal aging process. This highlights the need for anti-aging treatments to address age-related decline in vestibular function.

In recent years, several studies have investigated anti-aging interventions using drugs. The antioxidant coenzyme Q10 (CoQ10) is a typical drug studied for its anti-aging effects. Aging is partially attributed to reactive oxygen species (ROS) generated within the mitochondria, which damage key molecules such as mitochondrial DNA. CoQ10 exerts its anti-aging effects by inhibiting ROS generation [8,9]. In addition to its antioxidant properties, water-soluble CoQ10 is believed to exhibit neuroprotective effects by stabilizing mitochondrial membranes during oxidative stress [10]. Furthermore, oral administration of

* Corresponding author. Nara Medical University Otolaryngology, Head and Neck Surgery, Shijo-cho, Kashihara City, Nara Prefecture, 634-8522 840, Japan.
E-mail address: kamyk-j0@naramed-u.ac.jp (K. Ueda).

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CoQ10 has been shown to inhibit age-related changes in human inner ear HCs [11]. In mice, CoQ10 suppresses the expression of the apoptotic gene *Bak* and inhibits cochlear cell death, preventing age-related hearing loss [12].

Furthermore, Fujitsuka et al. demonstrated that, in a mouse model of aging, rikkunshito (RKT) reduced myocardial inflammation and hypothalamic microglial activity via elevated ghrelin and sirtuin1 (SIRT1) [13]. Ghrelin is a hormone secreted by the stomach during fasting that exerts anti-aging effects via the growth hormone secretagogue receptor type 1a [14]. Ghrelin and unacylated ghrelin stimulate human osteoblast growth in a capacity-dependent manner [15,16]. Therefore, RKT may inhibit age-related inflammation and bone density loss via ghrelin.

Despite these findings, few studies have investigated anti-aging treatments for age-related vestibular dysfunction.

In our previous studies, we demonstrated that aging decreased otolith density and function in mice [17]. In this study, we focused specifically on age-related vestibular disorders rather than on the general effects of aging. In the present study, we sought to investigate whether RKT and CoQ10, which have demonstrated anti-aging effects, could prevent the age-related loss of otolith density and function in mice.

2. Materials and methods

All procedures were performed in accordance with the guidelines outlined by the Ethics Committee for Animal Experiments of Nara Medical University, Nara, Japan.

3. Animals

Thirty male and 30 female 8-week-old C57BL/6 N mice were purchased from CLEA Japan (Tokyo, Japan) and bred in the Animal Experimentation Department of Nara Medical University. The mice were divided into three groups: control, CoQ10, and RKT groups. Each group comprised 10 male and 10 female mice. The control group was fed CE-2; the CoQ10 group was fed a special diet of CoQ10 (Fujifilm Ltd., Tokyo, Japan) mixed with CE-2 at 0.2 % concentration [18–20]; the RKT group was fed a special diet of RKT extract powder (Tsumura & Co., Tokyo, Japan) mixed with CE-2 at 3 % concentration [21]. RKT, a Japanese traditional Kampo medicine, was supplied in the form of a powdered extract (4.0g) obtained by spray-drying a hot water extract mixture of the following eight crude drugs: Atractylodis lanceae rhizoma (4.0 g), Ginseng radix (4.0 g), Pinelliae tuber (4.0 g), Poria (4.0 g), Zizyphi fructus (2.0 g), Citri unshiu pericarpium (2.0 g), Glycyrrhizae radix (1.0 g), and Zingiberis rhizoma (0.5 g).

All groups were reared under natural feeding conditions with special diets. Micro-computed tomography (μCT) and linear vestibulo-ocular reflex (LVOR) experiments were conducted at 80 weeks of age. Owing to natural mortality, the μCT experiment included 16 mice (32 ears) in the control group, 16 (32 ears) in the CoQ10 group, and 18 (36 ears) in the RKT group. The LVOR experiment for otoliths included 14 mice in the control group, 14 in the CoQ10 group, and 18 in the RKT group owing to white eyeballs and unclear images.

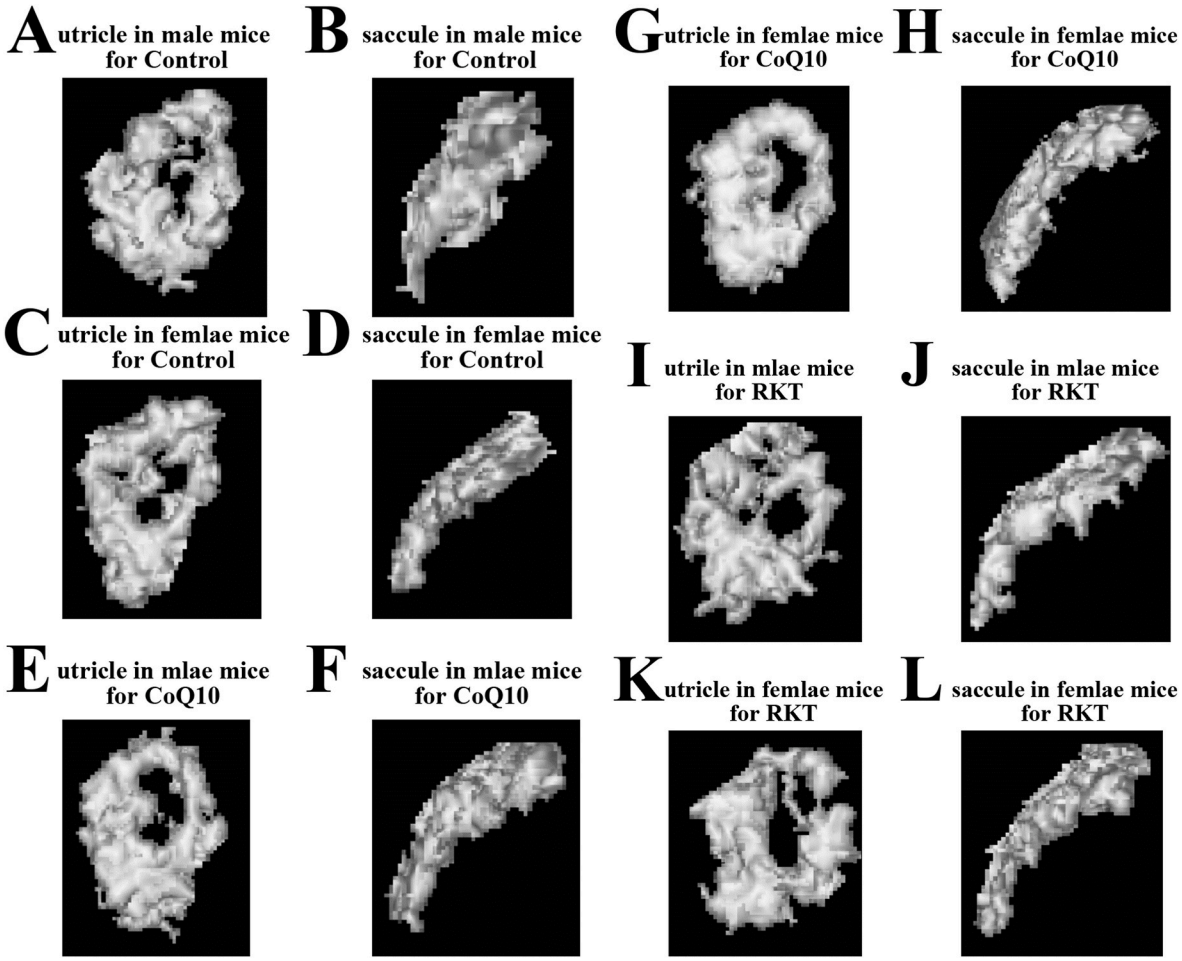

Fig. 1. Three-dimensional otolith models constructed from micro-computed tomography images.
Utricles are shown in the left column, while saccules are shown in the right column. **A–D**: the otoliths in mice fed with a control diet. **E–H**: the otoliths in mice fed with a special diet of coenzyme Q10. **I–L**: the otoliths in mice fed with a special diet of rikkunshito.

3.1. Evaluating morphological changes in the otoliths via three-dimensional μCT imaging

This experimental method was based on the previous study by Ueda et al. [17] (see Supplementary Material) and the main steps were as follows. First, mice were sedated by intraperitoneal anesthesia and placed in an animal holder, and the inner ear of each mouse was scanned using a CT scanner for animals. Second, we used a software called Attractive to identify the otolith organ area in the images and obtain a 3D model of the otolith (Fig. 1A–L). Attractive software automatically showed the volume and the average CT number of the 3D model of otolith.

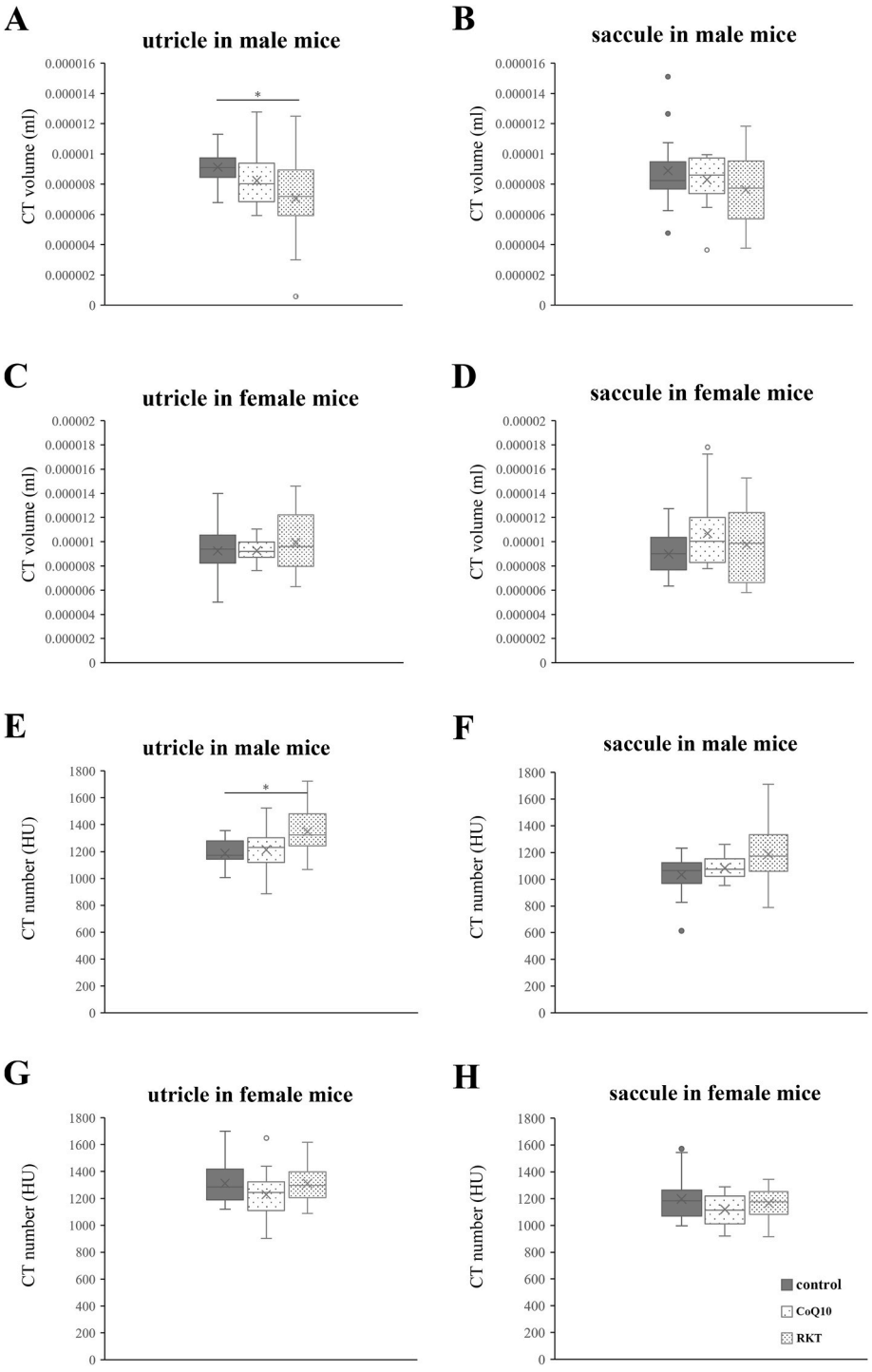

Fig. 2. Otolith analysis using micro-computed tomography of the control, coenzyme Q10 (CoQ10), and rikkunshito (RKT) groups.
A: utricle volumes in male mice; significant differences are observed between the control and RKT groups ($p = 0.0281$). **B**: saccule volumes in male mice; no significant differences are observed between any of the groups. **C**: utricle volumes in female mice; no significant differences are observed between any of the groups. **D**: saccule volumes in female mice; no significant differences are observed between any of the groups. **E**: utricle CT numbers in male mice; A significant difference is observed between the control and RKT groups ($p = 0.0185$). **F**: saccule CT numbers in male mice; no significant differences are observed between any of the groups. **G**: utricle CT numbers in female mice; no significant differences are observed between any of the groups. **H**: saccule CT numbers in female mice; no significant differences are observed between any of the groups.

For detailed experimental settings and parameters, such as the actual CT voltage, imaging time, slice thickness, CT manufacturer, and anesthetics used, please refer to the previous research paper [17]. However the age and diet of the mice were changed.

3.2. Evaluating functional changes in otoliths by analyzing the LVOR-induced eye movements

This experimental method was based on previous studies by Harada et al. [22] (see Supplementary Material), and the main steps were as follows. First, mice were sedated by intraperitoneal anesthesia. We incised the skin of the mice head and fixed a metal plate to the skull. Second, mice were placed on a device that could move in a reciprocating linear motion under computer control on a straight stainless rail. The mice were attached to the device by the metal plate on the skull. When the device moved on, linear acceleration was applied to the mouse and the eye movement was recorded by the high-speed camera on the device. This experiment was carried out in a dark room. Third, the eye movement images were analyzed using software developed by Imai, and the otolith function index was obtained.

For detailed experimental settings and parameters, such as the actual acceleration, the length of the stainless steel rail, and the anesthetic drugs used, please refer to previous research papers. However, the age and diet of the mice were different in the present study [17,22].

3.3. Statistical analyses

The Shapiro–Wilk test assessed the normality of all variables. If normality was confirmed, Bartlett's test was conducted to evaluate the variance. Welch's one-way analysis of variance (ANOVA), Bonferroni, Dunnett, and Steel tests were employed as post hoc tests to examine the effects of each drug.

If normality did not be confirmed, Steel test was performed. If normality and the homogeneity of variance across groups were confirmed, Dunnett test was performed. If the homogeneity of variance across groups was not confirmed, Welch's one-way ANOVA was performed.

All statistical analyses were performed using EZR (Saitama Medical Center, Jichi Medical University, Saitama, Japan), a graphical user interface for the R software (R Foundation for Statistical Computing, Vienna, Austria). EZR is a modified version of the R commander designed to add statistical functions that are commonly used in biostatistics [23]. Statistical significance was set at $p < 0.05$.

4. Results

4.1. Morphological changes in the otoliths due to drug administration

4.1.1. The volumes of the 3D models of otolith

In male mice, no significant difference was observed in utricle volume between the CoQ10 and control groups ($p = 0.106$, Steel test); however, a significant difference was found between the RKT and control groups ($p = 0.0281$, Steel test) (Fig. 2A). Regarding saccule volume, no significant differences were observed between any of the groups ($p = 0.557$, Welch's one-way ANOVA) (Fig. 2B).

In female mice, no significant difference was observed in utricle volume between the CoQ10 and control groups ($p = 0.632$, Dunnett test) or between the RKT and control groups ($p = 0.161$, Dunnett test) (Fig. 2C). Regarding saccule volume, no significant difference was observed between the CoQ10 and control groups ($p = 0.108$, Steel test) or between the RKT and control groups ($p = 0.792$, Steel test) (Fig. 2D).

Raw data is in supplementary data1.

4.1.2. The average CT numbers of the 3D models of otolith

In male mice, no significant difference was observed in the utricle CT number between the CoQ10 and control groups ($p = 0.863$, Dunnett test); however, a significant difference was found between the RKT and control groups ($p = 0.0104$, Dunnett test) (Fig. 2E). Regarding saccule volume, no significant differences were observed between any of the groups ($p = 0.0990$, Welch's one-way ANOVA) (Fig. 2F).

In female mice, no significant difference was observed in utricle volume between the CoQ10 and control groups ($p = 0.229$, Dunnett test) or between the RKT and control groups ($p = 1.00$, Dunnett test) (Fig. 2G). Regarding saccule volume, no significant difference was observed between the CoQ10 and control groups ($p = 0.154$, Dunnett test) or between the RKT and control groups ($p = 0.705$, Dunnett test) (Fig. 2H).

Raw data is in supplementary data1.

4.1.3. Funcitonal changes in the otoliths due to drug administration

In male mice: regarding lateral acceleration at 1.3 G, no significant difference was observed between the CoQ10 and control groups ($p = 0.853$, Dunnett test); however, a significant difference was found between the RKT and control groups ($p = 0.00681$, Dunnett test) (Fig. 3A). Regarding lateral acceleration at 0.7 G, no significant differences were observed between any of the group ($p = 0.321$, Welch's one-way ANOVA) (Fig. 3B). Regarding longitudinal acceleration at 1.3 G, no significant difference was observed between the CoQ10 and control groups ($p = 0.606$, Dunnett test); however, a significant difference was found between the RKT and control groups ($p = 0.0183$, Dunnett test) (Fig. 3C). Regarding longitudinal acceleration at 0.7 G, no significant difference was observed between the CoQ10 and control groups ($p = 0.841$, Dunnett test); however, a significant difference was found between the RKT and control groups ($p = 0.00322$, Dunnett test) (Fig. 3D).

In female mice: regarding lateral acceleration at 1.3 G, no significant difference was observed between the CoQ10 and control groups ($p = 0.673$, Dunnett test) or between the RKT and control groups ($p = 0.299$, Dunnett test) (Fig. 3E). Regarding lateral acceleration at 0.7 G, a significant difference was found between the CoQ10 and control groups ($p = 0.0133$, Steel test), but not between the RKT and control groups ($p = 0.934$, Steel test) (Fig. 3F). Regarding longitudinal acceleration at 1.3 G, no significant differences were observed between any of the groups ($p = 0.108$, Welch's one-way ANOVA) (Fig. 3G). Regarding longitudinal acceleration at 0.7 G, no significant difference was observed between the CoQ10 and control groups ($p = 0.867$, Dunnett test) or between the RKT and control groups ($p = 0.639$, Dunnett test) (Fig. 3H).

Raw data is in supplementary data2.

5. Discussion

In this study, we sought to investigate the efficacy of RKT and CoQ10 in preventing age-related structural and functional decline in the otoliths of mice. Analysis of the eye movements during linear acceleration showed that age-related decline in utricle function in female mice was prevented by the long-term oral CoQ10 administration (Fig. 3F). Furthermore, analysis of μCT images revealed that age-related utricular density decline in male mice was prevented by long-term oral RKT administration (Fig. 2E). These results indicate that prevention of both functional and morphological age-related otolith changes can be feasible.

5.1. The effect of coenzyme Q10 on otolith function

In this study, in female mice, the CoQ10 group exhibited a significantly stronger utricle-induced LVOR than the control group.

CoQ10 functions as an antioxidant and is known to reduce oxidative stress, enhance mitochondrial function, and decrease the expression of inflammatory genes. Someya et al. demonstrated that oral supplementation with mitochondrial antioxidants α-lipoic acid and coenzyme Q10 suppressed the expression of Bak, a mitochondrial pro-apoptotic gene, in the cochlea and reduced cochlear cell death in C57BL/6 mice [12]. Sato et al. also reported that CoQ10 effectively promotes recovery from

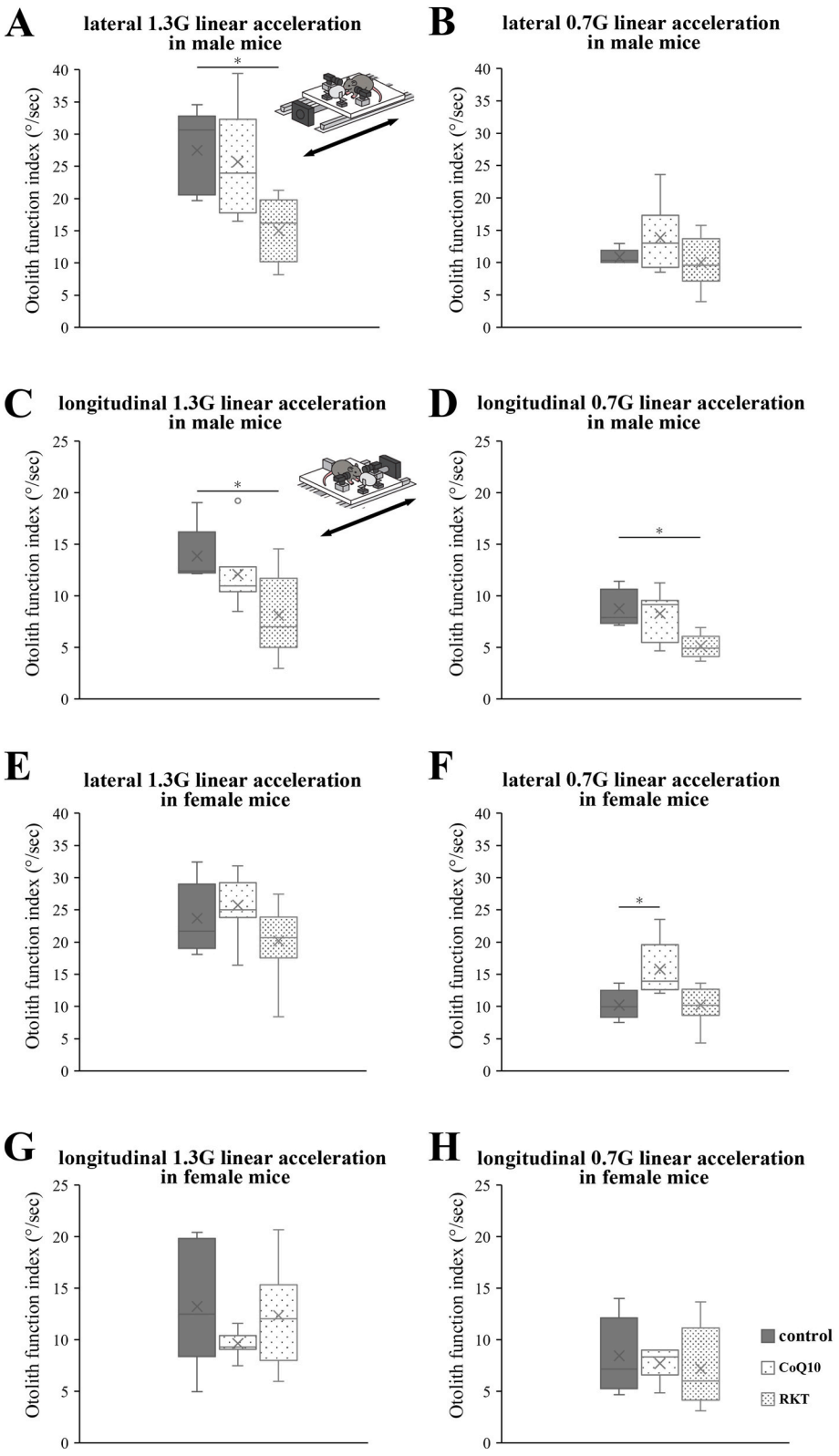

Fig. 3. Analysis of eye movements induced by the linear vestibulo–ocular reflex in the control, coenzyme Q10 (CoQ10), and rikkunshito (RKT) groups.
A: otolith function index during 1.3 G lateral acceleration in male mice; a significant difference is observed between the control and RKT groups ($p = 0.0117$). **B**: otolith function index during 0.7 G lateral acceleration in male mice; no significant differences are observed between any of the groups. **C**: otolith function index during 1.3 G longitudinal acceleration in male mice; no significant differences are observed between any of the groups. **D**: otolith function index during 0.7 G longitudinal acceleration in male mice; a significant difference is observed between the control and RKT groups ($p = 0.00510$). **E**: otolith function index during 1.3 G lateral acceleration in female mice; no significant differences are observed between any of the groups. **F**: otolith function index during 0.7 G lateral acceleration in female mice; a significant difference is observed between the control and CoQ10 groups ($p = 0.0133$). **G**: otolith function index during 1.3 G longitudinal acceleration in female mice; no significant differences are observed between any of the groups. **H**: otolith function index during 0.7 G longitudinal acceleration in female mice; no significant differences are observed between any of the group.

hypoxia-induced damage in auditory hair cells [24]. Sugahara et al. found that CoQ10 protects sensory hair cells from neomycin-induced cell death in mammalian vestibular epithelium, suggesting its potential as a protective therapeutic agent for the inner ear [25]. Müderris et al. administered approximately CoQ10 (500 μg/kg) to C57BL/6 mice (aged 12 weeks, n = 32), dissolving the compound in 30 % dimethyl sulfoxide and mixing it with drinking water. Their findings indicated reduced hearing threshold shifts, decreased expression of the pro-apoptotic gene Bak, lowered levels of inflammation-related genes NF-κB, COX-2, and iNOS, and increased expression of the anti-apoptotic genes Bcl-2 and Bcl-xL [26]. These results collectively support the conclusion that CoQ10 has a protective effect on the inner ear.

On the other hand, some research findings question the effectiveness of CoQ10. Sohal et al. reported that 14-week-old C57BL/6 mice were administered CoQ10 (93 mg or 371 mg/kg of body weight); however, there was no effect on antioxidant defense production or lifespan [19]. Similarly, Çeçen et al. found that administering CoQ10 to rats with vestibular nerve transection did not result in a significant difference in the compensatory process compared to the control group [27]. Given the differences in animal species, administration methods, and dosages, direct comparisons with results are challenging. Either way, the effects of CoQ10 should be carefully evaluated.

5.2. The effect of rikkunshito on otolith morphology and function

In this study, male mice in the RKT group had significantly greater utricle density than those in the control group.

Rats with bilateral ovariectomy-induced osteopenia/osteoporosis have decreased otolith density [28]. Thus, osteoporosis reduces otolith density. RKT induces ghrelin secretion from the stomach; ghrelin is known to proliferate osteoblasts, potentially preventing bone density loss [13,15,16,29], ultimately preventing osteoporosis. Therefore, RKT may prevent osteoporosis, and consequently otolith density loss, by inducing ghrelin secretion. Ghrelin signaling also activates the SIRT1 pathway, which has been suggested to protect against age-related inflammatory and apoptotic changes in the brain and heart [13]. Therefore, the combined effect of increased ghrelin secretion and SIRT1 activation via oral RKT administration may prevent inflammation and apoptosis and maintain otolith density.

In male mice, the RKT group had a significantly smaller volume of the utricular 3D CT model than control group. The increased utricular CT number and decreased volume of utricular 3D CT model suggest that while the density of the otolith was relatively maintained, its morphology may have changed. In fact, when comparing the 3D CT models of the utricle, the morphology appeared more heterogeneous in the RKT group compared to the control and CoQ10 groups (Fig. 1). The otolith organ is divided into a central region called striola and a peripheral region called extrastriola. In addition, otoliths exist on a gelatinous layer called the otolith membrane, and it is known that there are proteins associated with otoliths (*Oc90, Otolin-1, Osteopontin, α-Tectorin*) and proteins associated with the otolith membrane (*Otolin-1, Otogelin, Otoancorin, α-Tectorin, β-Tectorin*) [30]. It is possible that RKT influenced these components, leading to morphological changes in the otolith organ.

Regarding to otolith function, the RKT group had a significantly weaker LVOR. This result can be explained to some extent by morphological changes in the otoliths. The striola and extrastriola exhibit distinct characteristics in terms of hair cell types and immunostaining patterns. And a structure known as the line of polarity is also recognized in otolith. At this boundary, the kinocilia and stereocilia of hair cells are arranged facing away from each other on the other side of the line of polarity, suggesting potential interactions between them.

Considering these structural characteristics, the increased density of the otolith resulted in the morphological changes leading to otoconia accumulation in undesired areas, such as the striola. This redistribution of otoconia likely reduced the appropriate input stimuli to the hair cells, resulting in a lower VOR gain. While RKT may enhance otolith density, it may not have been sufficiently effective in regulating its spatial distribution.

Additionally, the RKT group exhibited significantly greater body weight than the control group (Supplementary Fig. 5). This weight gain is believed to be a result of increased appetite due to ghrelin activity induced by RKT. However, several studies have reported the adverse effects of obesity on physiological functions [31–33], which may be one of the contributing factors to the decreased otolith function. On the other hand, the VOR pathway are considered to include primary nerves, vestibular nuclei, vestibular cerebellum, extraocular muscles, and so on. So further research is needed into the interactions between those and RKT.

The discussion above included not only conclusions drawn from the results of this study but also hypotheses based on findings from other papers.

In any case, this study revealed that anti-aging treatments may be effective to a certain extent in treating age-related vestibular dysfunction. Anti-aging research is being conducted from various angles, including the use of various other drugs, exercise, calorie restriction, improvement of mitochondrial function, removal of senescent cells, and GLP-1, and further developments are expected in the future.

6. Limitations

6.1. The characteristic differentiation related to types of mice

We used C57BL/6 N mice in this study to maintain consistency with our previous study demonstrating age-related changes in otoliths [17]. It is worth noting that the nicotinamide nucleotide transhydrogenase (*NNT*) gene is functionally deleted in C57BL/6J mice, a close relative of C57BL/6 N, but not in C57BL/6JN mice [34]. NNT is located in the inner mitochondrial membrane and catalyzes the reduction of NADP + to NADPH; its presence enhances ROS detoxification. Therefore, in this study using C57BL/6 N mice, the effect of CoQ10 may have been stronger than that observed in other studies using C57BL/6J mice.

6.2. Drug administration

This study examined the effects of long-term drug administration. To minimize the stress and difficulty of continuous oral administration of the from the age of 8–80 weeks, the mice were fed a special diet mixed with the drug for spontaneous consumption. However, the actual amount of drug ingested by each mouse was unknown because of this method of administration.

6.3. The influence of drug administration for mice growth

In this experiment, mice began consuming the special diet containing the drug at 8 weeks of age, which could potentially affect their growth. Lundberg et al. reported that otolith seeding begins at E14.5, and growth begins immediately after seeding and continues until approximately one week after birth [30]. Additionally, Faulstich et al. stated that gaze-stabilizing eye movements in mice, including the VOR, reach full maturity by 3–4 weeks after birth [35]. Based on these findings, it is assumed that by 8 weeks of age, the morphology of the otolith organs and the functions related to the VOR evaluated in this experiment are fully developed. However, no studies have directly examined the effects of the special diet on overall growth, leaving its potential impact uncertain.

6.4. Drug concentration in plasma

Information on plasma levels in treated animals was not measured in this study. Yan et al. reported that Supplementation of SAMP1 female mice with CoQ10 for one week resulted in a plasma CoQ10

concentration 15.7 times higher than that of the control group [20]. No studies have reported the plasma concentration of RKT in mice. However, Kitagawa et al. provided a detailed analysis of RKT plasma concentrations in humans [36]. In their study, 18β-glycyrrhetinic acid showed the highest Cmax at a dose of 7.5 g, followed by atractylodin carboxylic acid and naringenin. The atractylodin acid and 18β-glycyrrhetinic acid showed a dose-dependent increase in blood concentration. Additionally, 18β-glycyrrhetinic acid was found to be slowly absorbed and remained in the body for a relatively long duration. Notably, atractylodin has been reported to have a protective effect on osteoblasts [37,38].

However, it is important to note that plasma concentrations were not measured in this study.

6.5. Indicators of anti-aging therapy

This study focused specifically on age-related vestibular dysfunction rather than the general effects of aging. Consequently, we did not assess DNA methylation patterns, telomere length, autophagy activity, or proteostasis, which are commonly used as established biomarkers for evaluating potential general anti-aging therapies.

Hypothesis. on the protective effect of CoQ10 against age-related otolith functional changes

As described in method section, we assessed otolith function by analyzing the eye movements during LVOR. Because the LVOR is the eye movements for stabilizing gaze during linear acceleration, the LVOR is influenced by several factors; the morphology and quantity of otoliths, HCs, primary nerves, vestibular nuclei, and so on. Possibly, CoQ10 may have a protective effect on some of these pathways.

In female mice, the otolith function index at 0.7 G was significantly different between the control and CoQ10 groups; however, the otolith function index at 1.3 G was not significantly greater in the CoQ10 group than in control group. This indicates that the response to lower-frequency stimuli was preserved by the oral administration of CoQ10. To understand this results, it is necessary to know how high-frequency and low-frequency stimuli are distinguished and perceived. In utricle, a central concavity, known as striola, is distinguished from the utricular periphery, the extrastriola. HCs in the inner ear are classified as type 1 HCs with large calyceal endings, and type 2 HCs with small bouton endings [39]. In mammals, type 1 and type 2 HCs are present in both the striola and extrastriola, with shorter kinocilia in the former and longer kinocilia in the latter [40]. Owing to differences in kinocilium length and the different displacement operating ranges of the HC receptors, the striola is thought to be more receptive to high-frequency stimuli, whereas the extrastriola is thought to be more receptive to lower-frequency stimuli [39,41–43].

Nam et al. reported that in the striola, the otolithic membrane is thin, while the gel layer is thick [40]. This characteristic is particularly pronounced in mammals, and the most common observation is that, in the striola, hair bundles often insert into or attach to the walls of canals (channels, holes, and pores) in the gel layer. Patients of advanced years generally showed a thickening of both the saccular and utricular otolithic membranes. Sometimes many or most of the residual otoconia were buried in its substance [44].

In addition, the formation or regeneration of the striola, hair cell, calyx, neural cell structures is considered to be regulated by genes such as *Cyp26b1, Neurod1,* Lgr5*, Sox2, Cib2, PLCγ, and Tmc1/Tmc2* [43, 45–50]. These genes play essential roles in various processes, including hair cell differentiation and maintenance, stem cell function, and the establishment of neural control via TrkB, Aldh1a3, and other factors.

It is possible that CoQ10 administration suppresses the thinning of the gelatinous membrane and mitigates damage to these genes, thereby preserving the striola structure and preventing the age-related decline in response to low-frequency stimulation.

7. Conclusion

CoQ10 prevents age-related decline in utricular function, and RKT may prevent age-related decline in utricular density. These findings suggest the potential clinical utility of these drugs for mitigating age-related balance disorders. However, further research is warranted to investigate the precise mechanisms by which CoQ10 and RKT exert their effects on otoliths, including its mechanism of action, and exploring the effects of these drugs in older mice to confirm reproducibility.

CRediT authorship contribution statement

Keita Ueda: Writing – original draft, Visualization, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Takao Imai:** Writing – review & editing, Validation, Supervision, Software, Project administration, Investigation, Formal analysis, Data curation, Conceptualization. **Tadao Okayasu:** Supervision, Project administration, Methodology, Investigation, Data curation, Conceptualization. **Tatsuhide Tanaka:** Supervision, Project administration, Methodology, Investigation, Conceptualization. **Kouko Tatsumi:** Supervision, Project administration, Methodology, Investigation, Conceptualization. **Akio Wanaka:** Supervision, Project administration, Methodology, Investigation, Conceptualization. **Tadashi Kitahara:** Writing – review & editing, Supervision, Project administration, Methodology, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Keita Ueda reports financial support was provided by Tsumura and Co. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bbrep.2025.102033.

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