**INTRODUCTION**

The incidence of hearing loss (HL) increases with aging, although several factors may accelerate its development in younger populations. Epidemiological studies have shown an association between deficiencies in essential nutrients and HL deficits that are quite common in the elderly. HL correlates with low serum folate levels and the derived systemic hyperhomocysteinemia (HHcy). Additionally, an inverse association between high plasma omega-3 polyunsaturated fatty acid (ω3) levels and age-related hearing loss (ARHL) was also described. HHcy is also related to vascular disease and might be the underlying cause of microvascular damage leading to the atrophy of the spiral vasculature, which is the cochlear structure that maintains its metabolic homeostasis. Based on these observations it was proposed that HHcy could be a risk factor for the development of HL, whereas nutritional interventions may have the potential to prevent ARHL.

**OBJECTIVES**

1. Evaluation of the impact of folic acid deficiency in HL.
2. Determination of the potential of nutritional ω3 supplementation to ameliorate HL.

**METHODS**

**Animals and diets:** Two-month old C57BL/6J and CBA/Ca mice were randomly divided in several groups according to the experiment. Experiment 1: C57BL/6J mice (N=65 each group) were fed a standard diet (normal folate 2 mg/kg, A04/A04C/R04 diet; Panlab/Safe) or a folate deficient (FD) diet (folate acid ≤ 0.1-0.2 mg/kg, Harlan Teklad TD.95247) for 2 months. Experiment 2: CBA/Ca mice (N=10 each group) were fed standard or FD diets for 8 months. Experiment 3: C57BL/6J mice were fed a standard diet including 40 g/kg soybean oil (control; N=15) or a standard diet with 38.5 g/kg of soybean oil and 1.5 g/kg of Eupoly-F97® EPA fish oil (ω3 group; N=9) for 8 months. All diets contained mineral mix (TD.94049 PWD Mineral Mix,AIN-93M-MX) and vitamin mix (TD.94047 PWD Vitamin Mix, AIN-93-VX) from Harlan Teklad. **Hearing assessment:** Auditory brainstem response (ABR) to broadband click and 4, 8, 16, 20, 28, and 40 kHz pure tone frequencies was analyzed. Recording of distortion product otoacoustic emissions (DPOAE) was performed after stimulation with f1 and f2 primary tones, with a ratio f2/f1 = 1.2. Primary tones for 8, 10, 14, 18 and 22 kHz frequencies were tested. **Expression, protein and metabolite levels:** Plasma total homocysteine (tHcy) and folate levels were measured by HPLC and a microbiological method, respectively. Total cochlear RNA was used to determine expression levels by RT-qPCR. Protein levels were analyzed by Western blotting in cochlear extracts. **Statistical analysis:** Results were analyzed with the Students t-test or ANOVA with Bonferroni pos-hoc test using IBM SPSS 22 software.

**RESULTS**

Hearing is affected by the intake of essential nutrients and the effect depends on the genetic background.

**C57BL/6J mice Prone to hearing loss**

<table>
<thead>
<tr>
<th>Age (months)</th>
<th>CBA/Ca mice Resistant to hearing loss</th>
</tr>
</thead>
<tbody>
<tr>
<td>2 months old</td>
<td>Normal ABR at the start of the experiments</td>
</tr>
<tr>
<td>7 months old</td>
<td>Disruption of cochlear structure in the FS group</td>
</tr>
<tr>
<td>10 months old</td>
<td>Impairment of cochlear Hcy metabolism</td>
</tr>
<tr>
<td>2 months old</td>
<td>Signs of cochlear oxidative stress in FD mice</td>
</tr>
<tr>
<td>7 months old</td>
<td>Neutral ABR at the start of the experiments</td>
</tr>
<tr>
<td>10 months old</td>
<td>No signs of hearing loss are observed after 5 months on a FD diet by ABR</td>
</tr>
</tbody>
</table>

**CONCLUSIONS**

- Progression of age-related hearing loss is affected by the folate intake, an effects that depends on the genetic background.
- Folate deficient diets cause disruption of the cochlear structure, impairment of homocysteine metabolism and oxidative stress.
- Omega 3 supplementation prevents most changes in homocysteine metabolism and inflammation induced by mice aging.

**Acknowledgements:**

- FP7-HEALTH-INNOVATION2-AFHELO, FP7-PEOPLE-TARGET