Induced Short-Term Hearing Loss due to Stimulation of Age-Related Factors by Intermittent Hypoxia, High-Fat Diet, and Galactose Injection

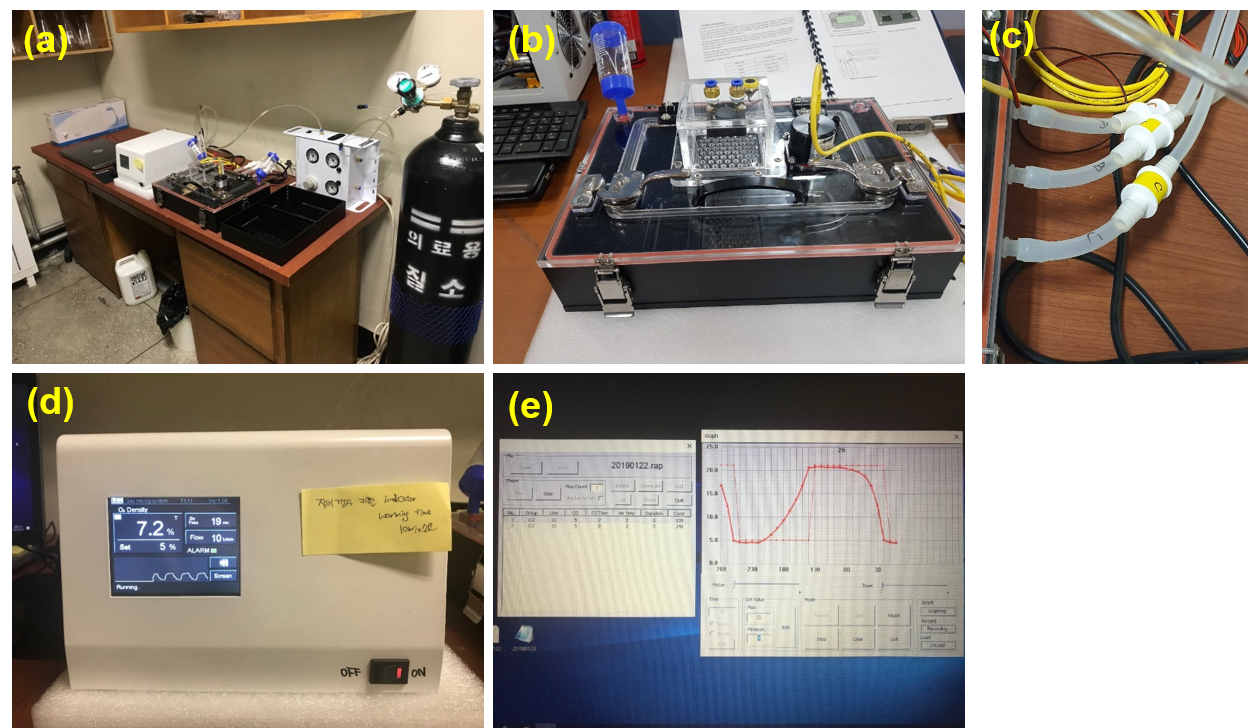
**Dong Jun Park 1, Sunmok Ha 2, Jin Sil Choi 1, Su Hoon Lee1, Jeong-Eun Park 3 and Young Joon Seo 1,\***

1 Department of Otorhinolaryngology, Yonsei University Wonju College of Medicine, 20 Ilsan-ro, Wonju, Gangwon-do 26426, South Korea.

2 Department of Biomedical Laboratory Science, College of Health Sciences, Yonsei University, wonju, Republic of Korea.

3 Department of Otorhinolaryngology Head and Neck Surgery, Hallym University College of Medicine, Dongtan Sacred Heart Hospital, Hwaseong, South Korea.

**\*** Correspondence: okas2000@hanmail.net; Tel.: +82-33-741-0644 (Y.J.S.)



**Figure S1.** Overall appearance of the hypoxic chamber.

(a) Installation of hypoxic chamber with nitrogen tank, (b) Appearance of hypoxic chamber, (c) Nitrogen injection and discharge nozzle, (d) Oxygen level meter in the hypoxic chamber, (e) Measurement of oxygen change in the hypoxic chamber.

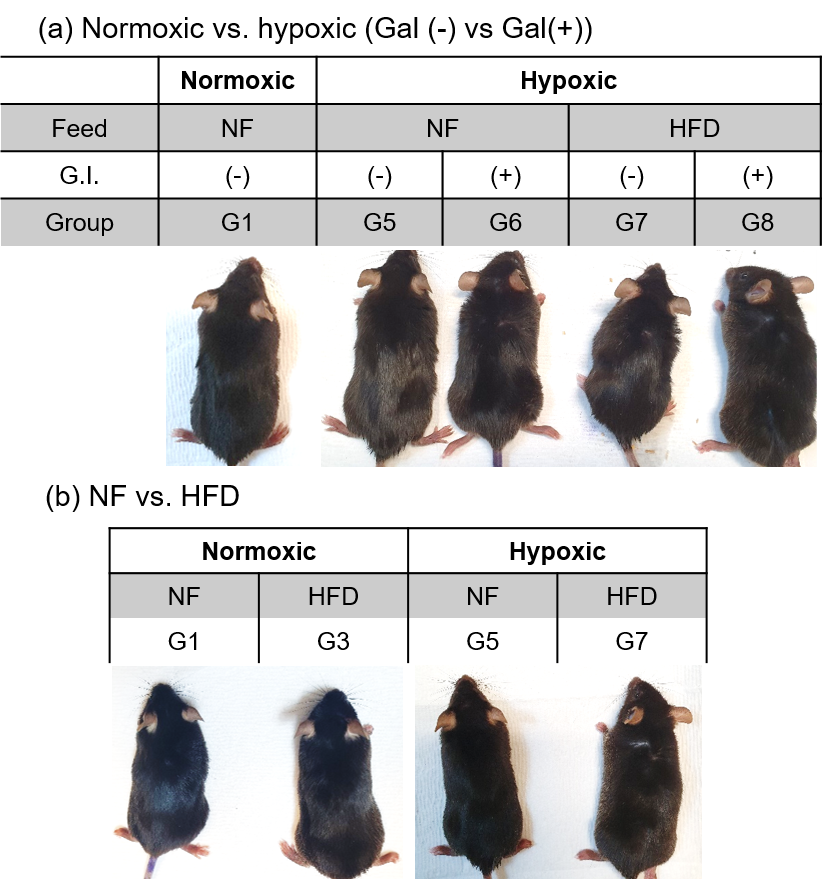


Figure S2. Phenotype appearance of mice groups (HFD, galactose injection, Hypoxic). (a) it showed the appearance of mice group on hypoxic group with galactose injection. (b) it showed the appearance of mice group on HFD in different air condition.

**Table S1.** Ingredient Composition of High-Fat Diet 32 (HFD32).

|  |  |
| --- | --- |
| Ingredient Composition | % per weight |
| Milk casein | 24.5 |
| Egg white | 5 |
| L-cystine | 0.43 |
| Powdered beef tallow  (including 80% of beef tallow) | 15.88 |
| Safflower oil (high oleic acid) | 20 |
| Crystalline Cellulose | 5.5 |
| Maltodextrin | 8.25 |
| Lactose | 6.928 |
| Sucrose | 6.75 |
| AIN93 vitamin mix | 1.4 |
| AIN93G mineral mix | 5 |
| Choline bitartrate | 0.36 |
| Teriary butylhydroquinone | 0.002 |
| Total | 100 |

**Table S2.** Ingredients and nutrient composition in experimental chows (NIH-41).

|  |  |
| --- | --- |
| Ingredient (Unit) | % per weight |
| Ground whole wheat | 34.9 |
| Ground No. 2 Yellow Corn | 21.0 |
| Ground whole oats | 10.0 |
| Wheat Middlings | 10.0 |
| Fish Meal (60% protein) | 9.0 |
| Soybean Meal (45% protein) | 5.0 |
| Soy Oil | 2.0 |
| Alfalfa Meal (17% protein) | 2.0 |
| Corn gluten meal (60% protein) | 2.0 |
| Dicalcium phosphate | 1.5 |
| Brewers dried east | 1.0 |
| Premixes | 0.6 |
| Limestone | 0.5 |
| Salt | 0.5 |
| Amino Acid Concentration | % of total diet |
| Arginine | 0.9 |
| Lysine | 0.85 |
| Methionine | 0.35 |
| Cystine | 0.25 |
| Tryptophan | 0.2 |
| Glycine | 0.95 |
| Histidine | 0.38 |
| Leucin | 1.4 |
| Isoleucine | 0.95 |
| Phenylalanine | 0.85 |
| Tyrosine | 0.6 |
| Threonine | 0.65 |
| Valine | 0.9 |
| Glycine | 0.95 |
| Mineral Concentration (Unit) | per weight |
| Calcium (%) | 1 |
| Phosphorous (%) | 0.94 |
| Potassium (%) | 0.55 |
| Sodium (%) | 0.25 |
| Magnesium (%) | 0.15 |
| Iron (ppm) | 300 |
| Zinc (ppm) | 40 |
| Manganese (ppm) | 140 |
| Copper (ppm) | 12 |
| Cobalt (ppm) | 0.7 |
| Iodine (ppm) | 1.8 |
| Vitamin Concentration (Unit) | per weight |
| Vitamin A (IU/g) | 17 |
| Vitamin D3 (IU/g) | 4 |
| Alpha-Tocopherol (IU/g) | 45 |
| Thiamine (ppm) | 15 |
| Riboflavin (ppm) | 9 |
| Niacin (ppm) | 70 |
| Pantothenic Acid (ppm) | 30 |
| Choline (ppm) | 1900 |
| Folic Acid (ppm) | 2 |
| Biotin (ppm) | 2 |
| Vitamin B12 (Mcg/kg) | 75 |
| Vitamin K (ppm) | 2 |

**Table S3.** Compared guaranteed analysis between NIH41 and HFD32 (%).

|  |  |  |
| --- | --- | --- |
| Guaranteed Analysis | NIH-41 | HFD32 |
| Moisture with vitamins | 36.9 | 6.2 |
| Crude Protein | 18.0 | 25.5 |
| Crude Fat | 5.0 | 32.0 |
| Crude Fiber | 5.0 | 2.9 |
| Ash (%) | 35.1 | 4.0 |
| NFE\* (%) | - | 29.4 |
| Total | 100 | 100 |

\*NFE = Nitrogen-Free extract

**Table S4.** Ingredient composition of AIN93-VX vitamin mix and AIN93G mineral mix.   
**a.** Ingredient composition of AIN93-VX vitamin mix

|  |  |
| --- | --- |
| Ingredient Composition | g per weight |
| Niacin | 3.0 |
| Calcium Pantothenate | 1.6 |
| Pyridoxine HCl | 0.7 |
| Thiamin (81%) | 0.6 |
| Riboflavin | 0.6 |
| Folic Acid | 0.2 |
| Biotin | 0.02 |
| Vitamin B12(0.1% in mannitol) | 2.5 |
| Vitamin E, DL-alpha tocopheryl acetate (500IU/g) | 15.0 |
| Vitamin A Palmitate (500,000IU/g) | 0.8 |
| Vitamin D3, Cholecalciferol (500,000 IU/g) | 0.2 |
| Vitamin K1, Phylloquinone | 0.075 |
| Sucrose, fine ground | 974.705 |
| Total | 1000 |

**b.** Ingredient composition of AIN93G mineral mix

|  |  |
| --- | --- |
| Ingredient Composition | g per weight |
| Calcium Carbonate | 357.0 |
| Potassium Phosphate | 196.0 |
| Potassium Citrate | 70.78 |
| Sodium Chloride | 74.0 |
| Potassium Sulfate | 46.6 |
| Magnesium Oxide | 24.3 |
| Ferric Citrate | 6.06 |
| Zinc Carbonate | 1.65 |
| Magnanous Carbonate | 0.63 |
| Cupric Carbonate | 0.31 |
| Potassium Lodate | 0.01 |
| Sodium Selenate | 0.0103 |
| Ammonium Paramolybdate | 0.008 |
| Sodium Meta-Silicate | 1.45 |
| Chromium Potassium Sulfate | 0.275 |
| Lithium Chloride | 0.0174 |
| Boric acid | 0.0815 |
| Sodium Fluoride | 0.0635 |
| Nickel Carbonate Hydroxide | 0.0318 |
| Ammonium Meta-vanadate | 0.0066 |
| Sucrose | 220.7159 |
| Total | 1000 |

**Table S5.** Thickness of the dermis and fat layers between different groups.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
|  | WT | +Gal | +HFD | +HFD, Gal | +Hypoxic | +Hypoxic, HFD, Gal |
| Dermis Thickness | 287.05±99.26 | 348.5±85.9 | 346.76±81.53 | 392.35±67.59 | 351.4±93.46 | 388.42±66.9 |
| Fat layer Thickness | 87.59±19.76 | 127.4±29.04 | 600.29±18.18 | 594.44±18.55 | 89.71±36.00 | 133.67±36.87 |

**Table S6.** Primers of target gene for aging.

|  |  |  |  |
| --- | --- | --- | --- |
| Target gene | Predicable  Dysfunctional effect | F/R | 5’ 🡪 3’ |
| *ApoE* | Age-related disorders,  Vascular disease | F | GGT TCG AGC CAA TAG TGG AA |
| R | ATG GAT GTT GTT GCA GGA CA |
| *EDN1* | Cardiovascular  complications | F | ACA CCG TCC TCT TCG TTT TG |
| R | GAG TC CTT GGA AAG TCA CG |
| *UCP2* | Mitochondria dysfunction | F | CTC AAA GCA GCC TCC AGA AC |
| R | ACA TCT GTG GCC TTG AAA CC |
| *CDH23* | Age-related  hearing loss | F | ATG GAG AGC CCT CTG GAA AT |
| R | ACC CAC AAA GGC TGT ACT GG |
| *KCNQ4* | Non-syndromic sensorineural hearing loss | F | TGT TGG GAT CCG TGG TCT AT |
| R | GAGTTG GCA TCC TTC TCA GC |
| *Myo7a* | Non-syndromic hearing loss and deafness | F | GAC AAC TCT AGC CGC TTT GG |
| R | GAC ACG TGA CTT CTC CAG CA |
| *Myo6* | Non-syndromic hearing loss and deafness | F | AGA CCA CTT CCG GCT CAC TA |
| R | TGG GTT GTC TCG TAG CAC AC |
| *SLC26A4* | Anion transport dysfunction | F | TCA TTG CCT TTG GGA TAA GC |
| R | GGC AAC CAT CAC AAT CAC AG |
| *18s* | Ribosomal RNA | F | CAT TCG AAC GTC TGC CCT AT |
| R | GTT TCT CAG GCT CCC TCT CC |