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# Impact of Chronic Simulated Snoring on Carotid Atherosclerosis in Rabbits

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**Background and Purpose** Chronic simulated snoring was induced in rabbits to determine the impact of snoring on the development of atherosclerosis.

**Methods** The pressure wave of induced snoring at the carotid bifurcation of rabbits was acquired by gently pressing the airway. This wave was then simulated using custom-made mechanical devices. Twelve rabbits were used in this study, seven of which were assigned to the experimental group and the remaining five formed the control group. All of the rabbits were raised on a 1% high-cholesterol diet. Either working or sham devices were positioned at the ventral center of the neck in each rabbit. At the end of a 2-month observation period, all of the rabbits were sacrificed by perfusion fixation, the carotid arteries harvested, and the carotid atherosclerosis histology reviewed.

**Results** All of the rabbits survived to the end of the experimental period. Blood sampling revealed the presence of hypercholesterolemia in both groups, with no significant difference between them. The presence and degree of atherosclerosis did not differ significantly between the groups.

**Conclusions** The findings of this study show the feasibility of making a chronic simulated snoring rabbit model. However, the causative role of snoring in carotid atherosclerosis was not detected in this animal study. **J Clin Neurol 2013;9:269-273**

**Key Words** induced snoring, carotid artery, atherosclerosis, rabbit, hypercholesterolemia.

## Introduction

Atherosclerosis and its consequences greatly affect human health and longevity.[1] The factors regarded as contributors or causes of vascular diseases include hyperlipidemia, hyperhomocysteinemia, metabolic derangements such as diabetes mellitus, turbulent flow, hypertension, mechanical injuries, immunological injuries, and obstructive sleep apnea syndrome.[2-4]

Snoring is a highly prevalent condition that occurs in 7-50% of people,[5-11] depending on their age, gender, ethnic group, and other relevant criteria. However, its significance has yet to be well defined. A recent epidemiological study found that the vibration associated with snoring is related to atherosclerosis of the carotid arteries.[12] That study found that the prevalence of atherosclerosis increased with the severity of snoring.

The aim of this study was to obtain direct evidence for a causal relationship between atherosclerosis and snoring. A chronic rabbit snoring model was devised and used under controlled conditions.

## Methods

### Subjects

This study was performed on female New Zealand White rabbits (2.5-3.0 kg). The study protocol was approved by the Institutional Animal Care and Use Committee of SMG-SNU Boramae Medical Center.

## Modeling of snoring

Anesthesia was induced in the rabbits by the intramuscular injection of Zoletil (3 mg/kg) and xylazine (5 mg/kg). Rabbits were positioned in a supine position and breathed spontaneously. After making a skin incision on the ventral side of the neck, the left carotid artery was exposed by blunt dissection up to the level of its bifurcation. A custom-designed balloon-tipped catheter (Department of Biomedical Engineering, Seoul National University Hospital) filled with water was placed beneath the carotid artery bifurcation. The catheter was connected to a pressure sensor (NovaSensor NPC-100, General Electric, Bently, NV, USA). The signal was amplified and filtered using a custom-built signal conditioning circuit and then digitized using a commercial data acquisition device (USB6009, National Instruments, Austin, TX, USA). The monitoring and analysis program was implemented in a visual programming language (LabVIEW, National Instruments).

Having obtained a baseline wave, a 20-g sandbag was placed on the trachea to induce snoring. The waveform of induced snoring was recorded. This procedure was conducted according to Amatoury et al.[13] The effect of snoring was simulated using a small DC vibrator motor with an eccentric rotor. A custom-built control circuit turned it on and off every 2 s (0.25 Hz with a 50% duty cycle) to mimic the normal respiratory pattern. The induced pressure waveform was recorded through the balloon-tipped catheter under the carotid bifurcation while applying the vibrator to the skin of the ventral neck. Visually, the obtained waveform was similar to that of induced snoring (Fig. 1). Four rabbits were used for this procedure. They were euthanized by the intravenous injection of urethane (1 g/kg; Sigma, St. Louis, MO, USA) and potassium chloride (2 mmol/kg).

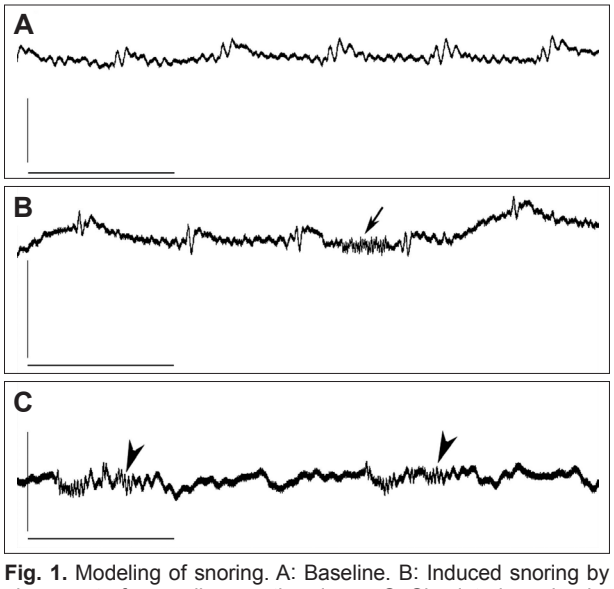


**Fig. 1.** Modeling of snoring. A: Baseline. B: Induced snoring by placement of a sandbag on the airway. C: Simulated snoring by intermittent motor activation. High-frequency waves caused by snoring are marked by arrows (B) and arrowheads (C). Horizontal bar=500 ms; vertical bar=1.5 mm Hg.

## Simulation of long-term snoring

The rabbits wore custom-made vests in order to simulate long-term snoring; the vest supported the vibrator so that it was in contact with the neck, and also provided pockets for the controller and batteries. The controller was programmed to operate for 12 h during the daytime and then stop for the following 12 h. The vibrating stimulus was applied from 6.00 a.m. to 6.00 p.m. to cover rabbit's usual sleeping time with some extension. Although it was possible to provide maximal stimulation (i.e., for the entire day), the animals were allowed some rest. Four AA batteries were used as a power source (Fig. 2). A total of 12 rabbits were assigned to two groups: experimental and control. The motor operated during the experimental period in the experimental group ($n$=7), while a sham device with a dummy motor and circuit board was installed in the vest in the control group ($n$=5), with no vibratory stimulation.

Rabbits were fed ad libitum with a high-cholesterol diet (1% cholesterol, DYET# 620007, Dyets, Bethlehem, PA, USA) and had free access to water. They were examined daily to ensure the correct positioning and proper operation of the apparatus. The batteries were changed weekly.

## Harvesting the carotid arteries

The carotid arteries were harvested for histological examination at the end of the 2-month experimental period. After the

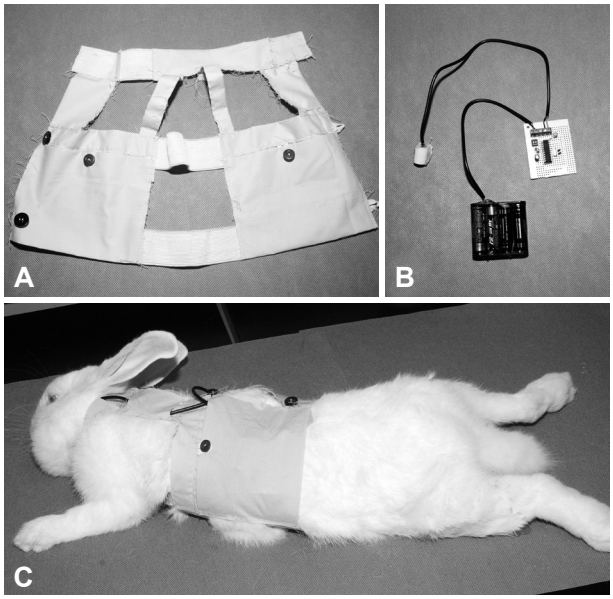


**Fig. 2.** A rabbit wearing a custom-made vest for long-term vibration simulation. A: The custom-made vest. B: Motor with a rotor, controller-mounted circuit board, and a battery pack. C: The fully equipped rabbit.

**Table 1.** Lipid panel findings for the experimental and control animals

| | Group | *n* | Mean (mg/dL) | SD (mg/dL) |
|---|---|---|---|---|
| Total cholesterol | Experimental | 7 | 1006.0 | 234.2 |
| | Control | 5 | 986.9 | 429.9 |
| Triglyceride | Experimental | 7 | 71.4 | 45.5 |
| | Control | 5 | 76.9 | 44.7 |
| HDL-C | Experimental | 7 | 23.8 | 4.7 |
| | Control | 5 | 26.0 | 5.7 |
| LDL-C | Experimental | 7 | 1054.0 | 205.5 |
| | Control | 5 | 1022.9 | 427.6 |

HDL-C: high-density lipoprotein cholesterol, LDL-C: low-density lipoprotein cholesterol, SD: standard deviation.

**Table 2.** Histological findings

| Rabbit no. | Group | Right | Left |
|---|---|---|---|
| 1 | Experimental | 1 | 0 |
| 2 | Experimental | 0 | 0 |
| 3 | Experimental | 1 | 2 |
| 4 | Experimental | 2 | 2 |
| 5 | Experimental | 1 | 1 |
| 6 | Experimental | 2 | 2 |
| 7 | Experimental | 2 | 1 |
| 8 | Control | 3 | 1 |
| 9 | Control | 2 | 2 |
| 10 | Control | 1 | 1 |
| 11 | Control | 2 | 0 |
| 12 | Control | 0 | 0 |

Modified American Heart Association classification.[14]

induction of anesthesia by the intramuscular injection of Zoletil and xylazine, as described above, urethane (1 g/kg) was injected intravenously for a deeper anesthesia. Peripheral blood was sampled from an ear vessel. A midline sternotomy was performed with preservation of the pericardium. All four cardiac chambers and great vessels were identified after incision of the pericardium, and cardiac puncture was performed at the apex of the left ventricle using an 18-G needle. Prewarmed Hartman solution was infused through the ventricular needle, and the right auricular appendage was opened with scissors for the simultaneous drainage of blood. After infusion of 500 mL of the solution, 4% cooled paraformaldehyde (about 250 mL) was infused through the ventricular needle. The right and left carotid arteries from 3 cm below to 1 cm after the bifurcation were harvested after neck dissection. Each harvested specimen was immersed separately and immediately in 4% paraformaldehyde.

### Histological examination

The harvested carotid arteries were fixed for 1 day and then embedded in paraffin. The specimens were subsequently sectioned at a thickness of 4 mm, mounted onto glass slides, and then stained with hematoxylin-eosin.

Atherosclerotic changes of the carotid artery were assessed by a pathologist according to the modified American Heart Association (AHA) classification suggested by Stary et al.[14,15]

### Statistical analysis

Statistical analysis was performed using SPSS software (version 14.0, SPSS Inc., Chicago, IL, USA). The independent-samples *t*-test and chi-square test were applied. Probability values of $p<0.05$ were considered statistically significant.

## Results

All of the rabbits survived to the end of the experimental period. The gross appearance of the harvested carotid arteries revealed no significant soft-tissue injuries or abnormal findings. The blood chemistry results revealed a similar level of hypercholesterolemia in all animals, irrespective of their grouping (Table 1).

Upon histological examination, each specimen exhibited a normal contour with a preserved lumen. Atherosclerotic changes were present in the carotid arteries from both groups, with no significant differences in the occurrence rates or degree of severity between them ($p=0.364$, chi-square test) (Table 2, Fig. 3).

## Discussion

Snoring is a highly prevalent condition[5-11] whose significance has not yet been well defined. Lee et al.[12] recently conducted an epidemiologic, observational cohort study following 110 subjects with or without snoring, and found that the prevalence of atherosclerosis increased with the severity of snoring. The authors suggested that the regional vibration may have been responsible for the carotid atherosclerosis. There have been no other similar studies. However, while the study of Lee et al. represents a novel and impressive interpretation of the epidemiological data, it has some shortcomings. For example, some of their subjects had accompanying obstructive sleep apnea syndrome, which is a well-known risk factor for atherosclerosis. Furthermore, their study provides only

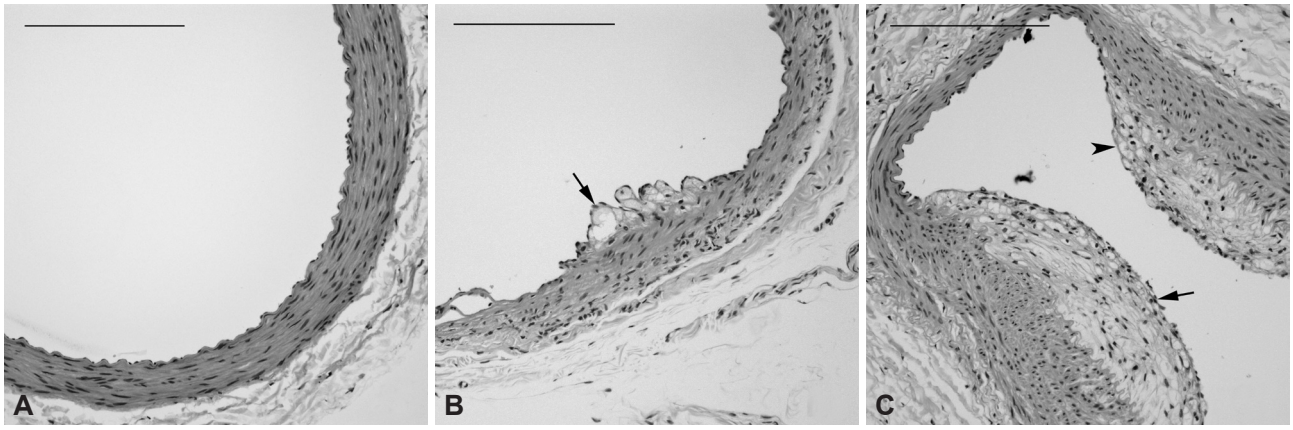


**Fig. 3.** Histologic findings of the harvested carotid arteries. A: Right carotid artery of rabbit #12 (control group) showing no definite atherosclerotic change. B: Right carotid artery of rabbit 35 (experimental group) showing focal infiltration of foamy cells under the intima (arrow). C: Right carotid artery of rabbit #8 (control group) showing multiple layers of foamy cell infiltration (arrow) with focal denudation of the intima (arrowhead). Hematoxylin-eosin stain. Scale bar=500 μm.

indirect evidence of the relationship between snoring and atherosclerosis due to the inherent limitations of observational studies.

The present study devised a chronic snoring rabbit model in an attempt to elucidate the significance of snoring without obstructive sleep apnea in the genesis of atheromas. The custom-built mechanical stimulating device could be applied to the rabbits in a noninvasive manner, such that it did not hurt the rabbit and enabled long-term maintenance. The use of a neck collar prevented the device from being influenced by the behavior of the rabbit.

The prevalence and severity of atherosclerosis did not differ significantly between the experimental and control groups in the present study. Although there were no positive findings, it would probably be impetuous to state categorically that snoring has no impact on the initiation or progression of carotid atherosclerosis. Several factors need to be considered when carrying out an experiment of this kind. Those factors are also the limitations of our study.

This study was subject to several limitations. First, the sample size may not have been sufficient to elucidate a cause-and-effect relationship. However, since there are no documented data on the prevalence of atherosclerosis in this condition, it is difficult to establish the ideal sample size. In addition, the optimal period of observation in this type of study has yet to be determined; for example, the impact of snoring on the initiation of atherosclerosis may require a shortening of the observation period. Furthermore, it was found that the aorta in rabbits consuming a 0.2% cholesterol diet exhibited foam cells and fatty streaks within 3-5 weeks.[16] This suggests that the experimental period in the present study was too long and that the cholesterol concentration in the rabbits' diet was too high to discern any difference between the groups. The implementation of a longer observation period could also have differentiated between them, since none of the present carotid specimens exhibited atherosclerosis beyond AHA stage 3. The short experimental period also prevented elucidation of the effect of the vibration other than intimal injury. Other authors have applied experimental periods of 6-60 months to identify advanced atherosclerosis.[17-22] Finally, although an attempt was made in this study to simulate real snoring, this is difficult to achieve precisely. Further refinement of the device will enhance the similarity between the model and real snoring.

To the best of our knowledge, this is the first report of the effect of long-term simulation of snoring for the development of atherosclerosis. Loudspeakers have been used by Puig et al.[23] to induce cell vibration and identify airway inflammation, by Almendros et al.[24] to trigger upper-airway inflammation in the rat, and by Cho et al.[25] to vibrate the carotid arteries of rabbits. In addition, Amatoury et al.[11] and Narayan et al.[26] induced snoring by placing a sandbag over the trachea. These examples were all *in vitro* or short-term *in vivo* models. The present study applied a noninvasive, simple, and reliable technique in rabbits, enabling the development of a new, long-term rabbit snoring model. Although the stimulus applied to the rabbit may not produce a precise simulation of snoring with respect to frequency, duration, and strength, it was an approximation of snoring with respect to the vibration transmitted to the carotid artery. It would be better if the parameters of the stimulus applied to each rabbit had been measured; however, this would have required an invasive procedure, which was not feasible in a long-term observation study.

## Conclusion

A long-term rabbit model of snoring was designed in this study. The obtained results did not reveal a causative effect of snoring on carotid atherosclerosis. Further investigation is

necessary to elucidate the possible causes of the negative findings of the present study. Changing the cholesterol content of the rabbits' diet and adjusting the study period may reveal the effect of snoring on the development of atherosclerosis.

## Conflicts of Interest

The authors have no financial conflicts of interest.

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