VIBROACOUSTIC DISEASE II: THE BIOLOGICAL AND ACOUSTICAL BASIS OF LOW FREQUENCY NOISE INDUCED PATHOLOGY

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1 INTRODUCTION

Vibroacoustic disease (VAD) is a whole-body pathology caused by long-term (in years) exposure to low frequency noise (LFN) (≤500 Hz, including infrasound)[1]. VAD has been identified in aircraft technicians[2], military[3] and commercial[4] pilots and cabin crew members. VAD has also been identified in a civilian population exposed to LFN due to military training exercises[5]. VAD is fundamentally characterized by the abnormal proliferation of the extra-cellular matrix. This feature is reflected in thickening of cardiac structures, namely the pericardium[6,7]. Pericardial thickening in the absence of an inflammatory process and with no diastolic dysfunction is the hallmark of this disease[8]. Table 1 summarizes the clinical stages of VAD. The goal of this report is to describe the basic aspects of VAD and LFN-exposure in animal models. Simultaneously, LFN-rich environments will be discussed as well as the deficiencies associated with the current noise legislation.

Table 1. Data corresponding to a group of 140 aircraft technicians (selected from an initial group of 306 workers), occupationally exposed to LFN. Exposure time refers to the amount of time it took for 70 individuals (50%) to develop the corresponding sign or symptom[1].

Clinical Stage	Sign/Symptom
Stage I - Mild (1-4 years)	Slight mood swings, Indigestion & heart-burn, Mouth/throat infections, Bronchitis
Stage II-Moderate (4-10 years)	Chest pain, Definite mood swings, Back pain, Fatigue, Fungal, viral and parasitic skin infections, Inflammation of stomach lining, Pain and blood in urine, Conjunctivitis, Allergies
Stage III – Severe (> 10 years)	Psychiatric disturbances, Haemorrhages of nasal, digestive and conjunctive mucosa [Small nose bleeds], Varicose veins and haemorrhoids, Duodenal ulcers, Spastic colitis, Decrease in visual acuity, Headaches, Severe joint pain, Intense muscular pain, Neurological disturbances

2 METHODS

The results of several studies are presented in this report. Thus, all applicable methodology will be described.

2.1 Noise Exposure and Noise Measurements

Figure 1 shows the frequency distribution of the acoustic environment to which the animal models were exposed.

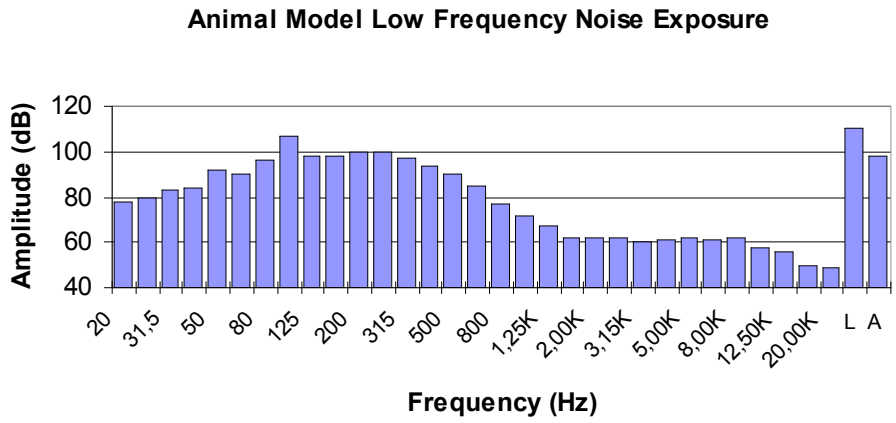

Fig. 1 A sound signal was generated by an analog noise generator, amplified and frequency filtered. The overall linear and A-weighted noise levels, as well as the spectral analysis of the excitation signal collected at the position near the rat test group inside the chamber is shown here. Noise was analyzed by a digital real time analyzer (B&K 2144). Acoustic energy is concentrated within the lower frequency bands due to the influence of the low-pass filter. In the frequency bands ranging from 50 Hz to 500 Hz the noise levels were larger than 90dB. The overall linear levels were above 109dB (L), and the A-weighted levels were 98dBA.

For the noise evaluations, sound pressure levels were measured with a modular precision sound level meter (B&K 2231). Frequency spectra were obtained using a real-time frequency analyzer (HP 3569A) in 1/3 octave frequency bands (from 6.3 Hz to 20000 Hz). Microphone calibration was achieved with a 250 Hz pistonphone (B&K 4228) to a sound pressure level of 124 dB re: 20 μPa. To expand the lower limiting frequency, the 1/2 inch microphone (B&K 4165) was attached with a coupler (B&K UC5265), thus permitting measurements to begin at 1.6 Hz

2.2 Animal Protocol

Wistar rats were fed standard rat food, had unrestrained access to water, and were treated in accordance with applicable legislation (86/609/CE). In one experiment, the study population was exposed to LFN on an occupationally-simulated schedule: 8 hrs/day, 5 days/week, weekends in silence. In another experiment. animals gestated and born within the LFN environment and then subsequently kept 1 year in silence or exposed to an additional 235, 2213, 4399 and 5304 cumulative hours of LFN. Control rats were kept in equal living conditions, but in continuous silence.

2.3 Electron Microscopy

All animals were sacrificed by a lethal intravenous injection of sodium-pentobarbital. The trachea was divided in two, along the saggital line. Specimens prepared for scanning electron microscopy (SEM) (JEOL JSM-35C) were dehydrated, critical point-dried, coated with gold-palladium and examined with the electron microscope at an accelerating voltage of 15 kV.

3 RESULTS OF ANIMAL STUDIES

3.1 The Low Frequency Noise Exposed Trachea

In Wistar rats, the respiratory tract epithelium is a target for LFN. Figs. 2 & 3 illustrate the damage incurred by this type of exposure. Ciliary fields are greatly depleted, and shaggy and sheared cilia

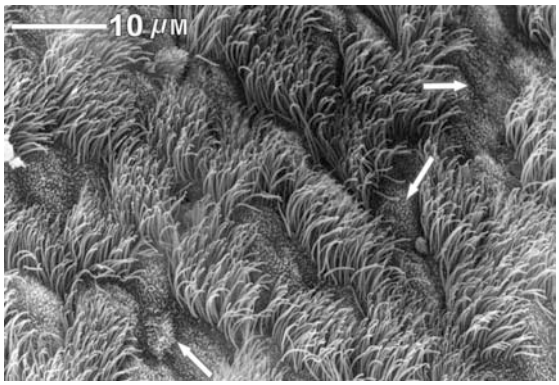

Fig. 2A SEM of control rat tracheal epithelium. Ciliary fields carpet the epithelium, interspersed with BCs (arrows). A ring of SC's surround each BC.

Fig. 3A SEM – control rat tracheal epithelium. Two BC (arrows) surrounded by rings of SC. BC microvilli are uniformly distributed over the surface.

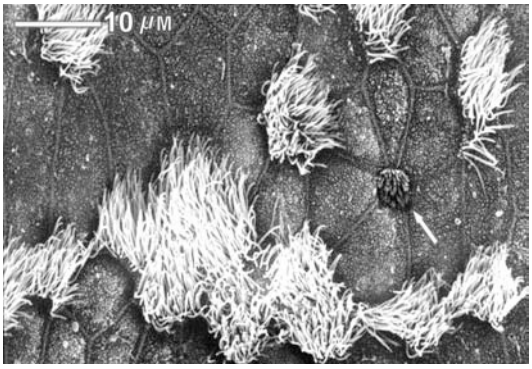

Fig. 2B SEM of rat gestated in LFN plus 1 year in silence. Ciliary fields are depleted. A rosetta-shaped structure centered on a BC (arrow) is evident.

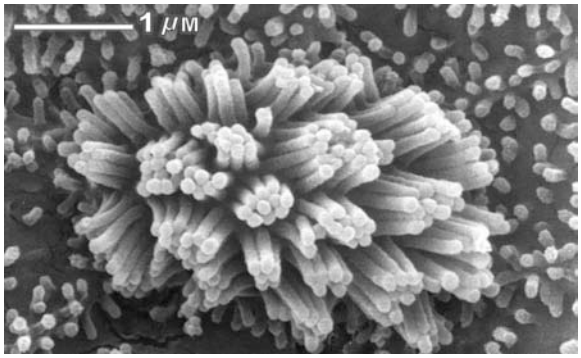

Fig. 3B SEM of rat tracheal epithelium exposed to 1984 hours of LFN. Amplification of a tracheal BC. Microvilli are clearly grouped together and, in some locations, appear almost fused.

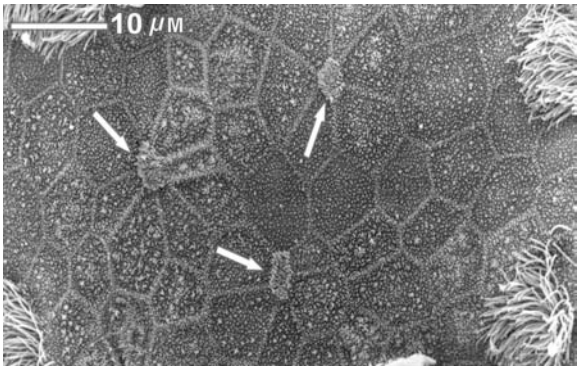

Fig. 2C. SEM of rat gestated in LFN + 2213 hrs of LFN. Ciliary fileds are depleted. BCs (arrows) are surrounded by rings of SCs. Intercellular junctions are thick and prominent.

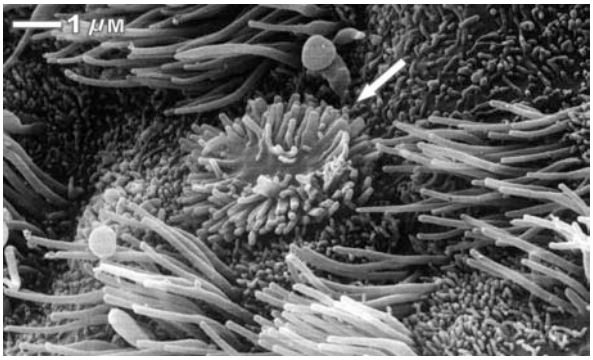

Fig. 3C SEM – exposed rat tracheal epithelium: 4399 hours of LFN. BC (arrow) microvilli are no longer regularly distributed. They appear fused and spreading outward from the center

are identifiable (Fig. 2B,C). Despite the damaged incurred by the cilia, their internal axoneme structure remains unaltered. Brush cells (BC) exhibit some of the most dramatic behavior. Normal BC has its microvilli uniformly distributed over its apical surface, which is open to the airway (Fig.3A). With LFN exposure, these structures tend to group together (Fig. 3B), and eventually fuse (Fig. 3C). Animals that are gestated within a LFN environment and, after birth, subsequently kept in silence for 1 year, still exhibit a dramatically damaged epithelium (Fig. 2B). With additional exposure after a LFN-gestation, the epithelium exhibits a metaplastic /displastic organization (Fig. 2C). Focal lung fibrosis was also observed in the respiratory tract of the exposed animals.

4. RESULTS OF ACOUSTICAL STUDIES

Within the scope of the VAD project, LFN was assessed at several locations. Environments with comparable dBA levels were selected, and their frequency distributions were compared, as well as the corresponding dBLin values. Table 2 summarizes the dBA and dBLin values obtained at the four different locations. Fig.4 shows the frequency distributions for the same four locations

Table 2. Summary of locations and measured sound pressure levels, with (dBA) and without (dBLin) the A-weighting system.

Location	dBA	dBLin
A340 Cockpit	72.1	83.2
Kitchen Restaurant	71.6	80.1
Stopped Train	71.4	92.0
Car at 120 km/h	71.2	100.8

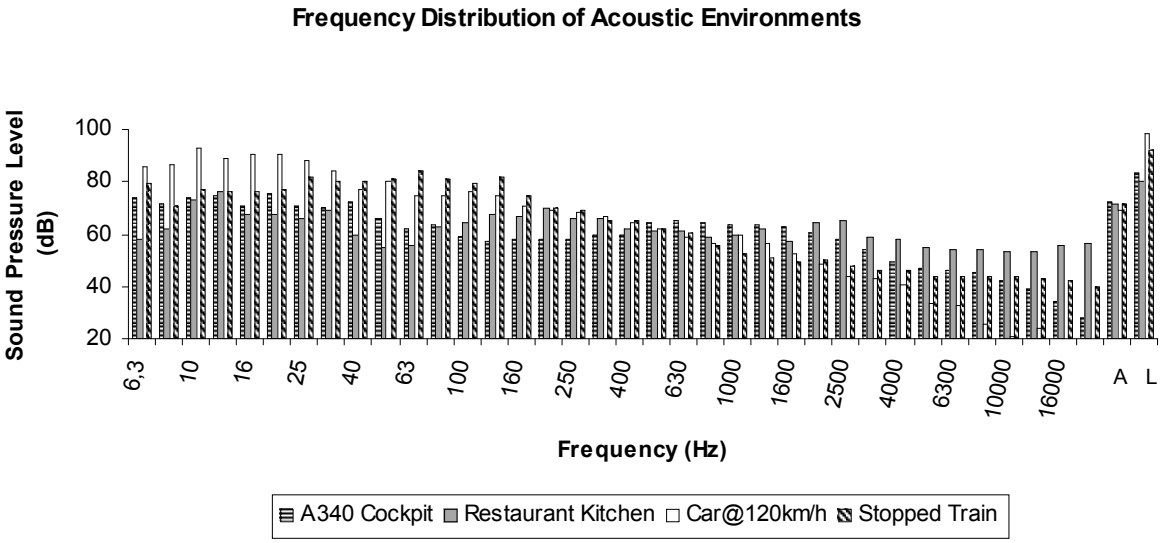

Fig. 4. Frequency distributions of the various acoustic environments. Cockpit of the Airbus 340, while at cruise flight. Restaurant kitchen during lunch hour. Car, a common passenger vehicle, alone on highway (at 3 a.m.), traveling at 120 km/h, with radio off and windows closed. Electric commuter train while stopped at the station. Energy is primarily concentrated below 500 Hz. dBA levels (A) are comparable. dBLin (L) levels are not.

At all locations, dBA levels are comparable, with a 0.9 dB difference between them. However, the actual acoustic content of these environments differ greatly, as is reflected by the dBLin

measurements. As can be seen in Fig. 4, much of the acoustic energy is concentrated within the lower frequency bands (≤500 Hz).

5. DISCUSSION

5.1. The Involvement of the Respiratory System

The function of the tracheal brush cell (BC), a unipolar cell also known as alveolar type III cell, remains unknown, although the possibility of secretory or receptor functions for the respiratory BC have been suggested[9,10]. BCs are distinguishable from other epithelial cells because they are non-ciliated and present an apical tuft of microvilli that are longer and thicker than in the adjacent cells[11,12] (See Fig.3A). Under LFN stress, these particular microvilli fuse (Fig. 3B). This is a consistent feature of the throughout the respiratory tract[13]. The mechanisms responsible for this microvilli fusion are unknown. However, there is reason to believe that actin-based structures may be a particular target for LFN stress. BC microvilli are composed of actin, as are the stereocilia found in the cochlea. Curiously, in LFN-exposed rodents, cochlear stereocilia also fuse, both among themselves and with the upper tectonic membrane. Unlike the non-exposed who lost cilia with the normal aging process, exposed rats lost no cilia[13].

The clinical implications of these features are, as yet, unknown. However, there is one electron microscopy aspect that is in accordance with known clinical information. In Fig 2C, the cellular organization exhibited by the squamous cells is one of de-diferentiation, strongly suggestive of metaplastic and displastic events. LFN has already been shown to be a genotoxic agent in both human[14,15] and animal[16] models. Cancer in VAD patients is not an uncommon, although there is a peculiarity: all respiratory tract tumors of VAD patients (7 smokers/ 3 non-smokers) are of one unique type - squamous cell carcinoma. All lung tumores (7 cases) are located in the upper right lobe[17].

The involvement of the respiratory tract during LFN exposure has been suggested for some time. The lung of dogs exposed to wideband noise at 105-155 dB exhibited small hemorrhages, 3mm in diameter, mostly in the upper right lobe. As the dB-level of the acoustic stimulus increased, the hemorrhages increased in number, but not in size[18]. Taken together with this information, the observation of focal lung fibrosis in both LFN-exposed human[19,20] and animal[21] models seems to be a significant factor.

Also in the 1960's, within the scope of the Apollo Space Program, volunteers were exposed to LFN concentrated around 100 Hz and at >100 dB, for 1-2 minutes[22,23]. All subjective responses were related to the respiratory system and included chest wall vibrations, gagging sensation, throat pressure and cough. One subjected coughed for 20 minutes after the stimulus had ceased to be present[22]. Curiously, in smokers and non-smokers alike, bronchitis is one of the symptoms that appear during the mild stage of VAD. Recently, a VAD patient employed for 10 years as a motorman described coughing onboard ships as a frequent occurrence, especially *after* leaving the engine rooms[24].

5.2 The Inadequacy of Standard Acoustic Measurements

Current legislation regarding noise is based on the erroneous assumption that acoustic phenomena only impinges on, or via, the auditory system[25]. Therefore, noise protection is solely equated with hearing protection. The A-weighting system is in place so that noise evaluations can better simulate human hearing, and thus prevent hearing impairment and deafness. The human auditory system captures sound within the 20-20,000 Hz range, however it is most sensitive within the 1000-10000 Hz range. (Professional deafness is established at the 4000 Hz notch.) Thus, the A-weighting system de-emphasizes the acoustic events that occur below 500 Hz. This is reflected in Fig. 4. Even though the dBA levels are comparable (See Table 2), because there is much acoustic energy

concentrated below 500 Hz, the real phenomena that is present (reflected by the dBLin values) differs significantly form the dBA value. In practice, this means that a dBA level measurement provides no information on the LFN content of the acoustic environment. In fact, having two environments of comparable dBA levels *does not equate to* having two acoustically similar environments (compare the frequency distributions of the Airbus 340 cockpit and the car in Fig. 4).

The implications of this for biomedical research are significant. It is well known that different organs possess different acoustic properties (impedance, resonance frequency, etc). Thus, exposing two populations to acoustic environments that have comparable dBA levels does not mean that one will necessarily obtain comparable results; in fact, results may be conflicting, as they often are[25]. The fact that the frequency distributions of these two environments are different (despite the comparable dBA level) can lead to entirely different results in otherwise comparable experiments.

To the extent of a postulation, this phenomenon has already been observed within the scope of the VAD project. Commercial airline pilots and flight attendants received echocardiograms in order to ascertain whether these professionals were also at risk for VAD[4]. The cockpits of airliners are generally considered to be quieter than the passenger cabins, considering the relative position of aircraft engines. However, contrary to what was expected, the pilots with same exposure time as flight attendants, had an accelerated rate of pericardial thickening. Detailed acoustic measurements of cockpits and passenger cabins of several commonly used aircraft models, revealed that there was a statistically significant difference in these two acoustic environments: cockpits had a much higher level of infrasound (<20 Hz)[26]. This excess infrasound was caused by the impact of airflow on the nose of the aircraft, and varied appropriately with speed and altitude[26]. It has thus been postulated that infrasound has a more specific effect on pericardial thickening than other LFN components.

6. CONCLUSIONS

LFN is an agent of disease that goes unchecked by current legislation. Long-term exposure to LFN can cause VAD. In LFN-exposed animal models, the respiratory tract seems to be a target for this acoustic stressor, and in LFN-exposed workers, respiratory pathology is frequent, in both smokers and non-smokers alike. The fact that most acoustic environments are described merely in terms of a dB-level measurement is a hindrance to biomedical research, since a dB-level measurement provides no information on the frequency content of the acoustic environment. Comparable dB-level measurements are not synonymous to comparable frequency distributions. In other words, a different predominance of specific frequency bands can yield different results in otherwise comparable experiments. Today, LFN is still not legally recognized as an agent of disease, and VAD is not legally recognized as a noise-induced pathology.

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