

# EXHIBIT 810

Review

# Vibroacoustic disease: Biological effects of infrasound and low-frequency noise explained by mechanotransduction cellular signalling

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Available online 4 August 2006

## Abstract

At present, infrasound (0–20 Hz) and low-frequency noise (20–500 Hz) (ILFN, 0–500 Hz) are agents of disease that go unchecked. Vibroacoustic disease (VAD) is a whole-body pathology that develops in individuals excessively exposed to ILFN. VAD has been diagnosed within several professional groups employed within the aeronautical industry, and in other heavy industries. However, given the ubiquitous nature of ILFN and the absence of legislation concerning ILFN, VAD is increasingly being diagnosed among members of the general population, including children. VAD is associated with the abnormal growth of extra-cellular matrices (collagen and elastin), in the absence of an inflammatory process. In VAD, the end-product of collagen and elastin growth is reinforcement of structural integrity. This is seen in blood vessels, cardiac structures, trachea, lung, and kidney of both VAD patients and ILFN-exposed animals. VAD is, essentially, a mechanotransduction disease. Inter- and intra-cellular communication is achieved through both biochemical and mechanotransduction signalling. When the structural components of tissue are altered, as is seen in ILFN-exposed specimens, the mechanically mediated signalling is, at best, impaired. Common medical diagnostic tests, such as EKG, EEG, as well as many blood chemistry analyses, are based on the mal-function of biochemical signalling processes. VAD patients typically present normal values for these tests. However, when echocardiography, brain MRI or histological studies are performed, where structural changes can be identified, all consistently show significant changes in VAD patients and ILFN-exposed animals. Frequency-specific effects are not yet known, valid dose-responses have been difficult to identify, and large-scale epidemiological studies are still lacking.

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*Keywords:* Extra-cellular matrix; Actin; Tubulin; Collagen; Tensegrity

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## 1. Introduction

This review paper deals with the biological effects of infrasound (0–20 Hz) and low-frequency noise (ILFN) (20–500 Hz). For the past 60 years, there has been much controversy and acrimonious debate over whether or not acoustical phenomena can cause extra-auditory effects on living organisms (Alves-Pereira, 1999). At present, the only officially (and legally) recognized consequence of noise exposure is hearing loss, albeit noise-induced annoyance, sleep disturbances and hypertension have been gaining more recognition over the past several years.

The scientific understanding of non-auditory, noise-induced biological effects can only be achieved if several obstacles are overcome. These obstacles pertain to the way the scientific community, in general, and biological scientists, in particular, view noise pollution and cellular signalling: noise only causes hearing impairment and cellular signalling is accomplished only through biochemical pathways. These untenable positions are powerful (scientific) hindrances that have impeded valuable research efforts. There are other key obstacles related to the awareness and recognition of ILFN as an agent of disease, but these are associated with the political, financial and social features of our collective societies and are, therefore, beyond the scope of this report.

Much of the literature pertaining to this field of study has been produced by non-English-speaking authors. Although the majority possess an abstract in English, full translations of all these scientific papers (e.g., from Chinese, Russian, Slovenian, Japanese and Polish) have been difficult to obtain. Additionally, many of the early papers produced by this team (from 1980 through 1989) were published in Portuguese with abstracts in English. Hence, several scientific papers in this review are only referred to abstracts.

Herein will be demonstrated that excessive exposure to ILFN causes extra-auditory pathology, specifically, vibroacoustic disease (VAD), and that the physiological and biological basis for this disease can only be understood if the concept of mechanotransduction cellular signalling is taken into account.

## 2. Noise pollution

Historically, it is understandable that noise exposure has always been associated with hearing loss. According to the Epic of Gilgamesh, a Babylonian king who lived in 2700 BC, the Great Flood was brought to

the planet Earth because the demi-gods were unable to sleep due to the noise produced by humans (Sandars, 1972). In Ancient Greece (600 BC), metalwork involving hammers was banned within city limits (Ward, 1973). In Ancient Rome, legislation existed pertaining to the noise associated with the iron wheels of wagons that disrupted sleep, while in certain cities of Medieval Europe, horse carriages were not allowed during night time (World Health Organization, 1999).

But with the advent of machines, a different kind of noise became ubiquitous. Sometimes, a low rumble from public transportation systems in urban and suburban settings, sometimes a hum from an air-conditioning unit, a refrigerator or a fan, this noise does not cause hearing impairment. But it may cause annoyance. As a definition of annoyance, the European Commission Noise Team (2000) maintains: “Annoyance is the scientific expression for the non-specific disturbance by noise, as reported in a structured field survey. Nearly every person that reports to be annoyed by noise in and around its home will also experience one or more of the following specific effects: Reduced enjoyment of balcony or garden; When inside the home with windows open: interference with sleep, communication, reading, watching television, listening to music and radio; Closing of bedroom windows in order to avoid sleep disturbance. Some of the persons that are annoyed by noise also experience one or more of the following effects: Sleep disturbance when windows and doors are closed; Interference with communication and other indoor activities when windows and doors are closed; Mental health effects; Noise-induced hearing impairment; Hypertension; Ischemic heart disease.” Hence, the parameter “annoyance” is, in itself, of a subjective nature.

### 2.1. *dBA versus dBLin*

Quantifying noise based on the subjective awareness of humans has guided the vast majority of noise-related biomedical studies. In fact, the foundational unit of noise legislation—the dBA—is grounded solely in the acoustical phenomena that humans can perceive with their ears, i.e., sound. The “A” on the dB unit refers to the usage of a filtering or weighting network that simulates human hearing and, thus, its purpose is to measure the acoustical phenomena present in the environment that can cause hearing impairment. Humans are considered to perceive sound between 20 and 20 000 Hz, but non-uniformly, i.e., there is an acoustical window where the human ear is most susceptible: 500–8000 Hz. It is within these frequency bands that hearing impairment occurs—legal deafness is assessed at 4000 Hz. Using the A-filter de-emphasizes all values of acoustical energy that occur below 500 Hz, and ignores all acoustical energy below 20 Hz. Fig. 1 demonstrates the usefulness of the dBA unit.

Scientifically, the acoustical environments of the cockpit and train shown in Fig. 1 cannot be considered comparable because the distribution of acoustical energy throughout the different frequency bands is quite distinct. This distinction is not taken into account if the acoustic environments are solely described by a dBA value, as is clearly illustrated in Fig. 1. Nevertheless, it is this dBA value that most noise-related legislation requires to assess the risk of noise exposure, and it is this same dBA value that most biomedical scientists use to describe their experimental acoustical environments.

The usage of the dBA value for scientific study can only be justified if the purpose of the study is related to effects on, or via, the human auditory system. If the purpose is to study the biological effects of noise, then describing acoustical environments merely in terms of a dBA value is a scientifically unsound methodology. Example: Two different and independent teams of researchers expose the same animal population, in equal conditions, to an acoustical environment described as “80 dBA”. Is it scientifically valid to compare the results of these two studies? As the example given in Fig. 1 clearly demonstrates, the answer is no, but the scientific community at large does so in its numerous published papers. At present, the general consensus of mainstream science is (still) that noise-induced extra-auditory pathology is a controversial, contradictory and inconclusive subject, hence non-existent and, therefore, not subjected to legislation (Alves-Pereira, 1999, 2005).

### 2.2. *What you can't hear, won't hurt you*

The title of this section is a quote, from Campanella (2001). There is no scientific evidence supporting this statement, and there is a colossal amount of scientific evidence indicating otherwise. Nevertheless, leading

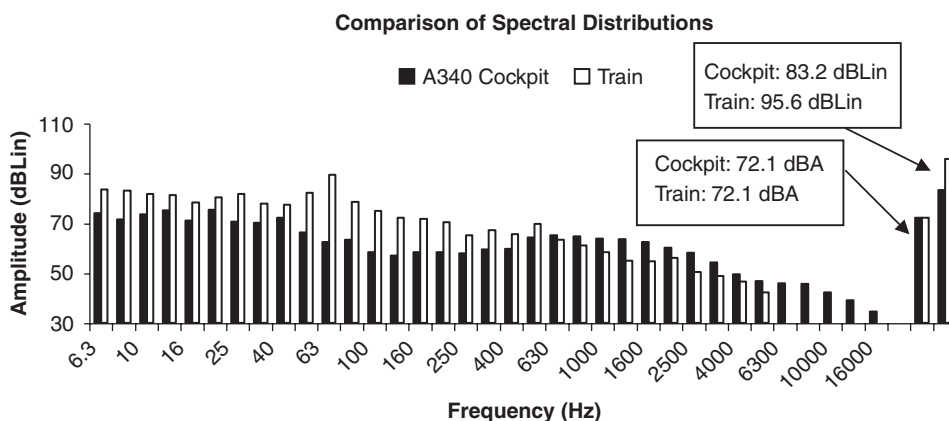


Fig. 1. Spectral distribution in 1/3 octave bands, without A-weighting (dB-Linear), obtained in (a) cockpit of the Airbus-340 (Alves-Pereira et al., 2001a), at cruise flight, and (b) in a Lisbon commuter train while in motion, in the passenger position (Alves-Pereira et al., 2004a). The two bars on the right compare the overall (Leq) values in both dBA and dBLin, i.e., with and without A-weighting, respectively. As can be observed, the dBA values are the same while the dBLin values differ by 12 dB. The value in dBA represents what the human can *hear* while the dBLin value indicates the amount of acoustical energy present in the environment. Hence, in both cockpit and train, what will be *heard* will be the same, but the individual in the train will be exposed to a larger amount of acoustical energy.

acousticians still opt to ignore this fact, and persist on perpetuating the notion that if hearing protectors are properly worn, no noise-induced extra-auditory effects will arise (von Gierke and Mohler, 2002).

In fact, over the past 25 years, research conducted in Portugal (and, independently, in other countries, such as the Soviet Union/Russia, Japan and China) has been showing that acoustical phenomena, whether it is perceived by the auditory system, or not, can indeed cause organic changes in biological tissue (Alves-Pereira, 1999). As a result of the efforts of a multidisciplinary team of scientists, including medical doctors, mathematicians, physicists, biologists, engineers, and acousticians, a pathological entity has been defined—VAD (see below) (Castelo Branco and Rodriguez Lopez, 1999a; Castelo Branco and Alves-Pereira, 2004a).

VAD is specifically caused by excessive exposure to ILFN (taken to be all acoustical phenomena occurring from 0 to 500 Hz). However, the acoustical energy responsible for VAD is never taken into account by standard noise measurements. As explained above, the scientific community and legislative bodies insist in accepting acoustical environments described merely in terms of a dBA value. Hence, they also perpetuate the erroneous notion that noise only affects the ear. The end result is a multitude of studies, focused on the biological effects of noise, but without the necessary methodology to allow them to be compared amongst each other. In the rare cases where information on the frequency distribution is provided, spectra are often measured in dBA units, which, once again de-emphasizes the amount of acoustical energy actually present in the environment, but gives a nice estimate of the noise being processed by the ear.

The scientifically unsubstantiated, but prevalent notion that noise only affects hearing has had a tremendous impact on individuals who develop ILFN-induced pathology. The gravity and magnitude of this issue will be further discussed in later sections.

### 2.3. Acoustic pollution

It is high time that scientists begin to view acoustical phenomena within a framework usually applied to electromagnetic phenomena. Within the electromagnetic spectrum, the human eye perceives light in a certain range of frequencies, just as within the acoustical spectrum, the human ear perceives sound in a specific range of frequencies. There exist electromagnetic phenomena that are not perceived by any of the human senses during the actual exposure (e.g. X-rays), and yet, excessive exposure to X-rays can cause severe biological damage. Without any subjective perception of the agent of disease, humans can nonetheless develop pathology caused by that unperceived agent of disease. While this is obviously true, it is apparently forgotten when one deals with acoustical phenomena, specifically ILFN. This may, in fact, be a unique case in the History of

Medicine, whereby the agent of disease is considered to only have an effect on the host, if the host perceives that same agent of disease.

That specific electromagnetic frequencies influence specific types of tissues is a well-known fact, and is the basis for numerous medical diagnostic and therapeutic tools. Science has data regarding which frequencies cause which types of pathology, for example, ultraviolet radiation can cause ocular disease, such as cataracts. But in the acoustical spectrum, the only frequencies that are considered to pathologically affect humans are within the audible range (ultrasound is beyond the scope of this paper), and all of them are focused on the hearing apparatus. Acoustical phenomena within the ILFN range can affect several organs and tissues, but this depends also on the frequency of the acoustical event because every organ and tissue has its own acoustical properties (e.g., resonance frequency and acoustical impedance).

Noise protection is, of course, focused exclusively on avoiding hearing impairment and minimizing annoyance. Thus, in some work environments where ILFN values can reach up to 90 and 100 dB (Alves-Pereira et al., 2004b), the only protection provided are hearing protectors. Returning to the analogy with the electromagnetic spectrum, this would be equivalent to providing dark glasses to individuals who work with X-rays.

Clearly, a new attitude toward noise and noise pollution is urgently required. The term *acoustic pollution* reflects the real nature of acoustical phenomena and how it impacts on humans. Acoustic pollution encompasses those frequencies that, being audible to humans, can cause hearing impairment. But it also includes all the other acoustical phenomena, ILFN and ultrasound, which may, or may not, pathologically affect human beings. Acoustic pollution deals with the entire acoustical landscape, and not just with the acoustical phenomena that produce sound to the human perception. In fact, where ILFN is concerned, dosimetry studies cannot be adequately carried out if the notion of acoustic pollution is not well understood. (See below.)

### 3. Chemical and mechanical cellular signalling

When new biological models successfully explain a larger number of biological events, usually that model is adopted and older, less applicable models are discarded. While this may seem like a logical course of action, sometimes the inertia associated with human nature to accept change constitutes an impediment.

The conventional model of the biological cell assumes it to be an elastic cortex surrounding a viscous cytoplasm that contains an elastic nucleus at the centre. This is a “continuum” model and assumes that the load-bearing elements are infinitesimally small compared to the overall size of the cell. Although this model has successfully explained many cellular behaviours, it does not take into account the distinct functional contributions of the cytoskeleton network.

For the past 30 years, the Ingber Laboratory at Harvard Medical School has been demonstrating that the “balloon” model of the cell is inadequate. Instead, a cellular model based on tensegrity architecture has been proposed and has been successfully explaining many cellular and tissue behaviours, both during normal metabolic activity and in disease (Ingber, 2003, 2004a, b, among others). This “new” cellular model is crucial to understanding the type of pathology developed by ILFN-exposed biological organisms because only the tensegrity model adequately explains how mechanical signals are transduced over cells and tissues.

At present, cell and tissue regulation is considered to be largely mediated by molecular conformations, intermolecular interactions, and linear signal transduction cascades. But this is proving to be a reductionist approach because the neglected mechanotransduction cellular signalling also plays a key role in cell and tissue communication (Ingber, 2004a). Modern medicine focuses on the importance of genes and chemical factors to control and explain tissue physiology and disease development. The “genome euphoria” (Ingber, 2003) disregards the physical (structural and mechanical) properties of cells and tissues despite the fact that in order to maintain normal cell behaviour (motility, growth, apoptosis), the ability of cells to sense and respond to mechanical stresses is of critical importance (e.g., Wang et al., 1993; Matthews et al., 2004; Alenghat et al., 2004).

#### 3.1. Tensegrity architecture

At the turn of the 20th century, mechanical interpretations of biological behaviour were a common methodology. Form and function of cells and tissues were of great importance to understanding biological

processes. However, as the field of molecular biology developed, biochemicals and genes became the forefront of scientific interest. “Medicine went from a holistic view of describing the relation between form and function to a much more reductionist view of describing what life is made of. And the mechanics were thrown out like the baby with the bathwater” (Ingber, 2004b).

The term *tensegrity* (tensile integrity) was coined by R. Buckminster Fuller, “father” of the geodesic dome in Architecture, and of the bucky ball in Physics (Fuller, 1975). Tensegrity is a form of structural stabilization that minimizes weight by using discontinuous-compression and continuous-tension, as opposed to continuous-compression. To visualize the difference, compare a brick-on-brick type of construction (continuous compression) with a stick-and-elastic construction of a geodesic dome, where the sticks are the discontinuous-compression elements while the elastics provide the continuous tension. Anchoring points, or nodes are essential to tensegrity structures because it is through these points that mechanical forces are transduced throughout the constituent compression and tensile elements. Any local, external perturbation of a tensegrity structure will result in a well-organized redistribution of tensional forces throughout the entire structure, with the purpose of maintaining structural integrity.

### 3.2. Cellular tensegrity architecture

Cellular cytoskeletons (CSK) form isomeric networks of microtubules, intermediate tubules, intermediate filaments, and actin. Forces generated within the CSK are involved in cytoplasmic organelle transportation (mitochondria and synaptic vesicles), chromosome movement during mitosis, and tension generation in the muscle cell contraction process (Ingber, 2003a). The CSK receives signalling from other cells through cell–cell junctions, and from the extra-cellular matrix (ECM), through cell–matrix junctions. Table 1 summarizes the properties of both types of junctions.

In the CSK, microfilaments form a mesh network of fine cables that constitute the continuous-tension elements (elastics) of the cellular tensegrity model. The compression elements (sticks) are formed by microtubules that are anchored to the ECM through transmembrane proteins called integrins, at sites called focal adhesions. Integrins differ from other cell-surface receptors because they bind with relatively low affinity ( $K_a = 10^6$ – $10^9$  l/mol), and their highest concentration is on cell surfaces.

Previous studies have probed the functioning of focal adhesion integrin receptors through magnetic twisting cytometry (Wang et al., 1993) and magnetic microneedle manipulation followed by magnetic pulling cytometry (Matthews et al., 2004). At focal adhesions, CSK and ECM possess structural linkages in the form of integrin cell-surface receptors. Mechanical forces applied directly to integrin cell-surface receptors alter cell biochemical and gene expression in a stress-dependent way (Ingber, 2003, 2004a, b; Wang, et al., 1993;

Table 1  
Properties of cell–cell anchoring junctions (adherens junction and desmosomes) and of cell–matrix anchoring junctions (focal adhesions and hemidesmosomes)

Junction	Cell–cell		Cell–matrix	
	Adherens junction	Desmosome	Focal adhesions	Hemidesmosomes
Intracellular (CSK) attachment filaments	Actin	Intermediate filaments	Actin	Intermediate filaments
Transmembrane adhesion protein	E-cadherin	Cadherin (desmoglein, desmocollin)	Integrin	Integrin ( $\alpha_6\beta_4$ , BP180)
Extracellular ligand	Cadherin (adjacent cell)	desmogleins, desmocollins (adjacent cell)	ECM proteins	ECM proteins
Intracellular anchor protein	$\alpha$ , $\beta$ -catenins, vinculin, $\alpha$ -actinin, $\gamma$ -catenin	desmoplakins, $\gamma$ -catenin	talin, vinculin, $\alpha$ -actinin, filamin	Plectin, BP230

Matthews et al., 2004; Alenghat et al., 2004). When the same forces are applied to other types of membrane receptors, there is no such effect.

External forces applied to integrins can activate intercellular signalling pathways, such as, protein tyrosine phosphorylation, ion fluxes, cAMP production, and G protein signalling (Ingber, 2003, 2004a, b; Wang, et al., 1993; Matthews et al., 2004; Alenghat et al., 2004). These integrin linkages allow for mechanochemical transduction signalling that produce changes in cell form and function. This type of intracellular signalling is a critical regulator of cellular biochemistry, gene expression and tissue development (Ingber, 2003, 2004a, b; Wang, et al., 1993; Matthews et al., 2004; Alenghat et al., 2004). There is a large variety of mechanochemical-transducing integrin receptors molecules; each type of integrin only binds to one ECM macromolecule, and cell-type specificity modulates integrin binding activities, i.e., in fibroblasts, ligands bind specifically to collagen, fibronectin and laminin. Hemidesmosomes (connecting the basal lamina to adjacent cells) are integrin receptors but that do not bind to the CSK through the actin cortex. Instead, hemidesmosome integrins connect directly with intermediate filaments. In the CSK, these are responsible for helping individual microtubules from buckling under compression, and link the nucleus to the surface membrane. Desmosomes connect intermediate filaments from cell to cell. The biological explanation for some diseases based on mechanotransduction impairment has already been successfully advanced (Ingber, 2003, 2004a, b).

#### 4. VAD

VAD is a systemic pathology, caused by excessive exposure to ILFN, and characterized by the abnormal proliferation of collagen and elastin, in the absence of an inflammatory process. VAD has been diagnosed in aeronautical technicians (Castelo Branco, 1999a), pilots and flight attendants (Araújo et al., 2001), as well as in an islander population exposed to environmental ILFN (Torres et al., 2001). Cases of VAD have also been documented among ship workers (Arnot, 2003) and in residential areas (Araújo et al., 2004, Monteiro et al., 2004).

##### 4.1. Brief chronology of scientific enquiry over the past 25 years

In 1980, co-author Castelo Branco was appointed chief medical officer at an aircraft manufacturing, repair and rework facility (OGMA), owned and operated by the Portuguese Air Force, and employing around 3500 workers. The first step was to visit the workstations of all employees to assess the nature of the different occupational hazards, possible emergency situations that could arise, and types of required worker protection.

After maintenance is performed on an aircraft, Quality Control personnel carry out manufacturer's procedures while the aircraft is stopped on the tarmac, and has its engines test run at all possible speeds. During one of these run-up tests (EA3B, with afterburn) Castelo Branco observed a worker beginning to walk aimlessly, without purpose, and in the direction of the turbines. A co-worker grabbed him by the arm before he got too close, and the incident remained at that. After the run-up test, the co-worker was questioned about what had happened. Apparently it was not a rare occurrence, and in the 1960s someone *had not* been caught in time, which led to a fatality. The non-purposeful movements exhibited by the worker appeared to Castelo Branco to be of an epileptic nature.

OGMA was founded in 1918 and, since the 1960s, detailed medical records are kept for all workers (administrative and technical). Based on the observation during the run-up test, the second step was to survey all medical records to count how many technicians had previously been diagnosed with late-onset epilepsy, as detailed in their medical files. In the Portuguese general population, the incidence of epilepsy is 0.2%. In the group of 306 aircraft technicians employed at OGMA, 10% had been previously diagnosed with late-onset epilepsy (GIMOGMA, 1984a). Here began this team's enquiry into ILFN-induced pathology.

##### 4.1.1. 1980

- Establishment that 10% ( $N = 306$ ) of the aircraft technicians employed at OGMA had been previously diagnosed with late-onset epilepsy (GIMOGMA, 1984a).

- Initiated neurophysiological examinations. The results from brainstem auditory evoked potentials (BAEP) were initially difficult to interpret, given their large dispersion. To better evaluate the BAEP recordings, taxonomic distances using clustering algorithms, and multivariate analysis of action currents distributions were applied, and a standardized method was developed using a control population (Castelo Branco et al., 1985; Marvão et al., 1985).

#### 4.1.2. 1984–1988

- Publication of the first articles on initial findings under the team name of GIMOGMA<sup>1</sup>: Epilepsy (GIMOGMA, 1984a), BAEP study (GIMOGMA, 1984b), and hyper-sensibility to noise (GIMOGMA, 1984c), otherwise known as noise intolerance or annoyance. A vascular involvement began to be suspected.

Until this point, it was thought that the neurological pathology observed in this group of workers, initially termed “vibration disease”, was due to excessive exposure to vibration. Neurological parameters continued to be assessed.

- Studies showed abnormal magnetic resonance imaging of the central nervous system (Cruz Maurício et al., 1988) and cognitive potentials (P300) (Moniz Botelho et al., 1988) in aircraft technicians.
- Other, non-neurological changes were identified, including damaged dental alveolar structures (Cortez-Pimentel and Castelo Branco, 1988); haemostasis and coagulation changes (Crespo et al., 1988), and abnormal retinal angiography (van Zeller et al., 1988). The latter two suggested the pathology was of a vascular nature.
- Four cases of pleural effusion developed in these workers, all of unknown aetiology. They exhibited an atypical response to standard therapeutics, and endured unusually prolonged recovery periods.

During these years, “systemic vibration disease” was the term used to identify the pathology observed in aircraft technicians (Pimenta et al., 1988). This meant that the health problems these workers were developing were not necessarily restricted to the neurological system.

- September 1987: Autopsy of an aircraft technician (Castelo Branco, 1999b). The plethora of scientific data bequeathed by this deceased patient disclosed the real extent of this pathology: 11 scars of previous silent infarct events, two previously undetected malignant tumours (kidney and brain), thickened blood vessel walls, thickened pericardium, and focal lung fibrosis.

#### 4.1.3. 1989–1992

During this period, it was determined that the fundamental agent of disease to which aircraft technicians were exposed was ILFN (Bento Coelho et al., 1994), hence, the pathological entity was, again, renamed: “whole-body noise and vibration syndrome” (Castelo Branco, 1992).

- The thickened blood vessels and pericardium found in autopsy prompted echo-imaging studies, namely echocardiography for assessing pericardial thickening. All aircraft technicians presented abnormal pericardial and/or cardiac valve thickening (Araújo et al., 1989).
- Carotid angiodynography was used to assess carotid thickening (Albuquerque et al., 1991; Carmo et al., 1992). Simultaneously, other populations occupationally exposed to ILFN began to be studied, such as helicopter (Carmo et al., 1992) and military (Canas et al., 1993) pilots.
- Wistar rats were chosen as animal models to investigate the effects of ILFN exposure on the respiratory tract, in an attempt to explain the atypical cases of pleural effusion, of unknown aetiology.

<sup>1</sup>GIMOGMA—Grupo de Investigação Médica das OGMA—was the acronym for the medical research team that began its activity at OGMA, and that today is represented by the Center for Human Performance.

#### 4.1.4. 1993–1999

In 1993, during a scientific meeting sponsored by our team, the term “vibroacoustic” was proposed for this pathological entity (Castelo Branco and Rodriguez Lopez, 1999a), and “vibroacoustic syndrome” became the new name for the ailment observed in aircraft technicians and, now, also in aircraft and helicopter pilots (Castelo Branco et al., 1996).

- Animal studies showed that the respiratory tract could be considered a primary target for ILFN: abnormal amount of fibrosis/collagen was ubiquitous in trachea, lungs and pleura; damaged (sheared) tracheal and bronchial cilia; fused actin-based microvilli of tracheal and bronchial brush cells (Sousa Pereira et al., 1999a; Grande et al., 1999). The atypical cases of pleural effusion were partially explained by morphofunctional impairment of pleural microvilli (Sousa Pereira et al., 1999b), as well as of pleural phagocytic capabilities (Oliveira et al., 1999).
- Additional neurological disorders were identified in ILFN-exposed populations, such as the existence of the palmo-mental reflex, usually only seen in primates, newborns, and the elderly (Martinho Pimenta et al., 1999a); balance disturbances (Martinho Pimenta et al., 1999b), and facial dyskinesia induced by auditory stimuli (Rosado et al., 1993; Martinho Pimenta and Castelo Branco, 1999c).
- The genotoxicity of ILFN was demonstrated in both human (Silva et al., 1999, 2002a) and animal models (Silva et al., 2002b), and was confirmed by teratogenic features in mice (Castelo Branco et al., 2003 g).
- Echocardiography was deemed an unreliable diagnostic tool because there is no established procedure to assess pericardial thickening and, thus, technician subjectivity introduced a large factor of error.
- The first human pericardial fragments were studied in VAD patients who required cardiac bypass surgery for other reasons (See below): abnormal amounts of collagen as well as the neo-formation of an extra layer of tissue was shown to be the cause underlying the pericardial thickening, providing anatomical confirmation of the autopsy and echo-imaging observations (Castelo Branco et al., 1996).
- Medical files of all aircraft technicians were chronologically reviewed since their admittance to OGMA. On-the-job accidents and incidents were correlated with the existence of unmonitored ILFN exposure of the workforce (Alvarez et al., 1993), and the clinical phases of the disease were outlined (Castelo Branco and Martinho Pimenta, 1995).

In 1999, the name “VAD” was adopted, and the journal *Aviation, Space & Environmental Medicine* dedicated a supplemental issue to this new pathological entity (Castelo Branco et al., 1999c).

#### 4.1.5. Since 2000

- Other ILFN-exposed professionals were studied, such as civil aviation pilots and cabin crewmembers, confirming echocardiography results of aircraft technicians and military pilots (Araújo et al., 2001).
- More neurological pathology was identified: VAD patients were found to be unable to hyperventilate when in the presence of excessive CO<sub>2</sub> (Reis Ferreira et al., 2003a).
- Mechanically induced cellular death was identified in the pericardia of VAD patients and it was hypothesized that this situation could be related to the large incidence of auto-immune disorders in these patients (Castelo Branco et al., 2004b).
- Further rat studies suggested that fusion of cochlear cilia (actin-based structures) may provide a biomechanical explanation for noise intolerance, or annoyance (Castelo Branco et al., 2003a).
- The first case of large-scale environmental exposure to ILFN appeared in Vieques, Puerto Rico (Torres et al., 2001). Here, ILFN was caused by military training exercises. An isolated case came from Dublin, Ireland, where buses where the source of ILFN and induced VAD in a home-maker (Monteiro et al., 2004). Another from Lisbon, where ship-to-silo and silo-to-ship loading of cereals produces ILFN in a home where both parents and 10-year-old child exhibited VAD-related signs and symptoms (Araújo et al., 2004).
- All bronchoscopic examinations of VAD patients disclosed lesions that, upon analysis, demonstrated the existence of abnormal amounts of collagen, and neo-formation of vascular beds. Disrupted collagen fibers were observed and correlated with a positive testing of anti-nuclear antibodies, providing a deeper understanding of auto-immune processes (Monteiro et al., 2004a).

#### 4.2. Clinical stages of VAD

In order to identify the clinical stages of VAD, as observed in aeronautical technicians, a systematic and detailed review of the medical files pertaining to the initial group of 306 aircraft technicians was undertaken in the mid 1990s. This group of 306 male individuals were all employed by OGMA for more than 10 years, and were submitted to rigorous selection criteria, as per Table 2 (Castelo Branco, 1999a).

A group of 140 technicians (average age of 42 years, SD = 10.4) remained after the application of selection criteria, i.e., 166 individuals were excluded. The medical files of these 140 technicians were comprehensively and chronologically reviewed. Simultaneously, a sociologist and a social worker interviewed family and friends to obtain additional information on the individual's behaviour outside his professional activity. The methodology to obtain a correspondence between sign/symptom and years of occupational exposure was the 50% cutoff, i.e., the sign/symptom was included in the list if it was identified in 50% ( $N = 70$ ) of the study population. Thus, referring to Table 2, after 1–4 years of occupational exposure, at least 70 of these 140 individuals developed bronchitis, in smokers and non-smokers alike (smokers in study group:  $N = 45$ ). Or, after 10 years of occupational activity, at least 70 exhibited headaches and nose bleeds. It should be emphasized that these signs and symptoms are not mutually exclusive, and most VAD patients suffer from more than one or two of these clinical situations, simultaneously (Castelo Branco, 1999a; Castelo Branco and Alves-Pereira, 2004a).

Table 3 refers to the signs and symptoms developed specifically by aircraft technicians working the standard 8 h/day, 5 days/week. Not all ILFN-exposed workers have this exposure schedule. For example, ship machinists can spend 3 weeks onboard ship (i.e., exposed to substantial ILFN-rich environments) and 2 weeks at home (i.e., presumably not in ILFN-rich environments) (Arnot, 2003). Other professional activities exist where the ILFN-exposure time pattern is not the standard 8-h/day exposure, such as with submarine and oil

Table 2  
Conditions for study population exclusion (Castelo Branco and Rodriguez Lopez, 1999a)

Conditions	Comments
Streptococcal infections	Due to their propensity to induce extra-cellular matrix changes
Diabetes mellitus	Same as above
Pre-existing cardiovascular disease	But not labile hypertension, because it is suspected to be a measure of individual susceptibility, and because lesions are distinct from those caused by established hypertension
Tobacco abuse	Smokers of more than 20 cigarettes a day
Alcohol abuse	Drinkers with more than a liter of wine per day (10–12% alcohol content)
Drug use	Users of any recreational or psychotropic drug

Table 3  
Data from a group of 140 aircraft technicians

Clinical stage	Sign/symptom
Stage I-Mild (1–4 years)	Slight mood swings, indigestion and 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, varicose veins and haemorrhoids, duodenal ulcers, spastic colitis, decrease in visual acuity, headaches, severe joint pain, intense muscular pain, neurological disturbances

ILFN exposure time (years) refers to the amount of time it took for 70 individuals (50%) to develop the corresponding sign or symptom (Castelo Branco, 1999a).

rig operators, astronauts, and environmental exposures in residential areas, where exposure can be continuous over long periods of time, and exists during sleeping hours. In these cases, the evolution of signs and symptoms could be greatly accelerated. For example, in the case of a Dublin homemaker, epileptic seizures consistent with VAD developed after 3 years of residence within a ILFN-infested home (Monteiro et al., 2004). If the ILFN exposure is environmental and/or leisurely, the standard 8 h/day model is also not applicable. Moreover, since different ILFN environments have unique frequency distributions, the fact that some frequency bands may be more predominant than others (i.e., concentrate more acoustical energy) can lead to the development of slightly different pathology.

#### 4.3. Pathology associated with VAD

Other important pathologies were identified among these 140 aircraft technicians, but since they were not identified in 50% of the population, they were not included in Table 3. Nevertheless, their incidence is clinically important. Some kind of respiratory insufficiency was found in 24 of the 140 professionals, 11 were smokers. In 10 of the 24 cases, a mere light physical effort was necessary to produce symptoms. Notably, only 45 of the 140 individuals were smokers, 38 of which had over 20 years of occupational ILFN exposure.

Late-onset epilepsy was diagnosed in 22 individuals, some of whom saw their seizures subside when away from their workstation. Reflex epilepsy due to vibratory stimulus (Martinho Pimenta and Castelo Branco, 1999c) and visual stimulus was observed in two individuals. Auditory stimuli did not trigger seizures but, in some cases, triggered rage reactions and movement disorders (Martinho Pimenta and Castelo Branco, 1999d, e). Balance disturbances were also a common complaint, identified in 80 individuals, although the severity of the balance disturbance ranged from dizziness to severe vertigo (Martinho Pimenta et al., 1999f). Unique and sudden episodes of non-convulsive neurological deficit occurred in 11 individuals. These were diagnosed as cerebral ischaemic vascular accidents, which was compatible with imaging studies. EEG and multi-modal evoked potentials showed considerable power changes that were in agreement with clinical psychological and neurological evidences. Delays in multi-modal evoked potentials (including endogenous), observed in all 140 patients, are a sign of progressive neurological deterioration and early aging process, as is the appearance of the archaic palmo-mental reflex, that affects about 40% of these 140 patients.

Endocrine disorders, the most common being thyroid dysfunction, were identified in 18 cases. The overall national Portuguese rate for adult thyroid dysfunction is 0.97% vs. the 12.8% identified in our group of 140 technicians. Similarly, diabetes was seen in 16 individuals (average age 39 years, SD = 7.8) (11.4%), while the overall national rate for a similar age-group is 4.6% (Castelo Branco, 1999a). Among the 140 professionals, 28 had malignant tumours. Five of these 28 individuals exhibited simultaneous tumours of different types. All CNS tumours ( $N = 5$ ) were malignant gliomata, and all respiratory system tumours were squamous cell carcinomas (5 in lung, 1 in larynx). To date, and to the authors' knowledge, a total of 11 VAD patients have developed respiratory tract tumours: 9 in the lung, and 2 in the glottis (3 smokers); all have been squamous cell carcinomas (Mendes et al., 2004; Reis Ferreira et al., 2005). Other tumours were found in the stomach ( $N = 10$ ), colon and rectum ( $N = 9$ ), soft tissue ( $N = 1$ ), and bladder ( $N = 1$ ) (Castelo Branco, 1999a). All digestive system tumours were low-differentiated adenocarcinomas. These data led to the investigation of the genotoxicity of ILFN. In both human (Silva et al., 1999, 2002a) and animal (Silva et al., 2002b) models, ILFN induced an increased frequency of sister chromatid exchanges, effectively demonstrating that ILFN is a genotoxic agent.

More recently, in 2003, a new pathological sign was identified among VAD patients: decreased respiratory drive (Reis Ferreira et al., 2003a; Castelo Branco et al., 2003b). To date, pulmonary function tests are normal in VAD patients, except the  $P_{0,1}(\text{CO}_2)$  index and the metacholine reactivity test. The  $P_{0,1}(\text{CO}_2)$  index is a measure of the inspiratory pressure (or suction) developed at the mouth, 0.1 s after the start of inspiration. This initial respiratory drive originates in the autonomic (or involuntary) pathway of the neural control of the respiratory function. By rebreathing  $\text{CO}_2$ , normal individuals would present a minimum six-fold increase of the  $P_{0,1}(\text{CO}_2)$  index when compared to normal  $P_{0,1}$ . If the neural control of respiration is compromised, then a less-than six-fold increase would be expected in the  $P_{0,1}(\text{CO}_2)$  index (Calverly, 1999; Cotes, 1993; Gibson, 1996). In VAD patients, all  $P_{0,1}(\text{CO}_2)$  index values are below 50%, when normal values would be above 60%.

Lastly, the issue of auto-immune diseases in ILFN-exposed individuals. In the electron microscopy studies of VAD-patient pericardial fragments, non-apoptotic cellular death was frequently observed (Castelo Branco et al., 2003c) (see below). Instead, biomechanical forces seemed to be responsible for the images of burst cells, with live organelles and no surrounding plasma membrane. Under these circumstances, the appearance of auto-immune diseases in these patients is not unreasonable. Indeed, previous studies have shown that ILFN exposure induces an accelerated onset of lupus in lupus-prone mice (Águas et al., 1999a). Lupus has also been identified in flight attendants (Araújo et al., 2001), and in entire families of islanders exposed to environmental ILFN (Torres et al., 2001). Vitiligo is another common finding, especially in the ILFN-exposed islander population. Vitiligo is associated with immune changes of CD8 and CD4 lymphocyte populations. These immune changes have also been observed in ILFN-exposed workers (Castro et al., 1999) and animal models (Águas et al., 1999b). Other authors have also corroborated the existence of auto-immune processes in noise-exposed workers (Matsumoto et al., 1989,1992; Jones et al., 1976; Soutar et al., 1974; Lippmann et al., 1973).

#### 4.4. *Some important considerations on behalf of VAD patients*

Legally, the only pathology that can develop due to excessive noise exposure is hearing impairment. Therefore, occupational physicians rarely view VAD symptomatology as caused by excessive noise exposure. In fact, given the plethora of complaints associated with VAD (see Table 3), oftentimes physicians regard the patient as a malingerer or hypochondriac (Castelo Branco and Rodríguez Lopez, 1999a), especially since routine medical tests (e.g., blood chemistry analysis, EKG and EEG) do not corroborate the existence of any pathology. One of the reasons for this is that the majority of medical diagnostic tests are based on biochemical, and not biomechanical, pathways (see below). There are dire consequences for the patients, as have been candidly exposed by a Scotsman, who was employed as a motorman, and developed VAD (Arnot, 2003).

In the case of occupational exposure to ILFN, workers can develop disabilities requiring early retirement (Castelo Branco et al., 1999d). Usually ILFN-rich environments are associated with machinery that, in an ever-developing technological world, often becomes obsolete within a few years time. At present, many individuals who have developed VAD due to occupational exposures cannot prove that they have been exposed to ILFN because noise assessments do not take ILFN into account (as described above), and many of the ILFN sources have been retired.

### 5. VAD in light of mechanobiology

The establishment of VAD has been problematic because of several, non-typical situations that seem to defy conventional medical concepts. For example, the production of collagen, in the absence of an inflammatory process, is consistently seen in the blood and lymphatic vessel walls (Castelo Branco et al., 1999b; Reis Ferreira et al., 2003b,c; Monteiro et al., 2004a), pericardium (Castelo Branco et al., 1999b, 2003c), trachea (Reis Ferreira et al., 2003b), and lung and pleura (Reis Ferreira et al., 2003c) of VAD patients. It is also observed in the respiratory tract (Castelo Branco et al., 2003a), kidney (Castelo Branco et al., 2003d), blood and lymphatic vessels (Martins dos Santos et al., 2002, 2004, respectively) of ILFN-exposed animals.

Much of the data collected on VAD and ILFN-exposed biological tissues has been in the form of ultrastructure micrographs, obtained with scanning (SEM) and transmission (TEM) electron microscopy. The following sections will describe the anatomical findings in VAD patients' pericardia, and in the respiratory tract and cochlea of ILFN-exposed rats, based on information obtained through histological and ultrastructural studies. The implications within the context of mechanobiology will be discussed.

#### 5.1. *The pericardium*

The pericardium is a fibrous sac that encases the heart, with the purpose of maintaining it in its normal position. External forces, due to respiration or changes in body posture, are absorbed by the pericardium so as to keep the heart and its cardiac rhythm intact. Consisting of three tissue layers—mesothelium, fibrosa and epipericardium—the pericardium is a highly organized mass of connective tissue, with a predominance of

collagen fibers arranged in accordion-like bundles. Elastic fibers, much less numerous than collagen fibers, intersect the collagen bundles at right angles. This anatomical arrangement taken together with the viscoelastic properties of both collagen and elastin, provide the pericardium with the mechanical capability of protecting the integrity of the cardiac cycle. The thickness of normal parietal leaflet of the pericardium is <0.5 mm (Shabetai, 1994). The mesothelium is in direct contact with the pericardial sac, and is formed by a one-layer thick sheet of mesothelial (cuboidal) cell (MC). Anchoring junctions interconnect MC among themselves, through their cytoskeletal fibers—microtubules—interconnected through desmosomes (see Table 1). Microtubules do not rupture when stretched, can withstand larger stresses and strains than actin filaments, and are crucial to maintain cellular integrity.

Abnormally thickened pericardia were first observed by this team in autopsy (Castelo Branco, 1999b), and later confirmed through echocardiography (Araújo et al., 1989; Marciniak et al., 1999; Torres et al., 2001). No inflammatory process was present, no cardiac dysfunction was identified and, thus, pericarditis is not an issue in VAD patients. The strange feature was that despite the extraordinary enlargement, no diastolic impairment was observed: EKGs of VAD patients were normal, as were the cardiac functional parameters assessed through echocardiography. Simultaneously, echo-imaging did not have a 1–1 correspondence with anatomical structures, thus, it became important to understand what was occurring at the anatomical level.

Since one of the consequences of ILFN exposure is thickening of blood vessel walls (Castelo Branco, 1999b; Castelo Branco et al., 1996, 1999b, 2003c; Reis Ferreira et al., 2003b,c) the recommendation for cardiac bypass surgery by other physicians is not uncommon among VAD patients. Hence, it was possible, with Hospital Ethics Committee approval and patients' fully informed consent, to study pericardial fragments of VAD patients (Castelo Branco et al., 1996, 1999b, 2004b). Pericardial thickening was confirmed anatomically, and a possible reason for the lack of diastolic dysfunction was uncovered (see Fig. 2).

When electron microscopy was used to examine these pericardial fragments, an unusual bursting of cells was observed. This peculiar type of cellular death was related to the mechanical bursting of cells, with images of seemingly live organelles outside of the burst cytoplasmic membrane (Figs. 3 and 5). Cellular debris

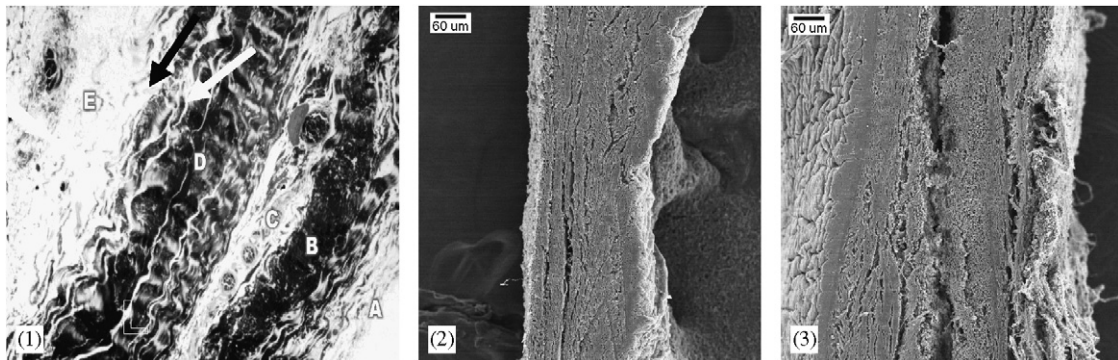


Fig. 2. (1) Light microscopy (100 ×)—VAD patient pericardium, with pericardial sac on right. Five (instead of the normal three) layers are identifiable: (A) mesothelial, (B) internal fibrosa, (C) loose tissue, (D) external fibrosa, and (E) epipericardium. The loose tissue is rich in vessels. No inflammatory cellularity was identified in any of the five layers. In both fibrous layers, wavy collagen bundles are visible, however the wave length of fibers in layer B (internal fibrosa) is smaller than that in layer D (external fibrosa). Taking together the increased amount of collagen bundles, in wavy, accordion-like arrangements, with different orientations in relation to each other, and with more than one elastic fiber accompanying the bundles at seemingly perpendicular angles (seen through electron microscopy, not shown), seems to suggest a pneumatic-like structure, designed to absorb abnormally large external forces. Similarly, this functional arrangement also explains why there is no diastolic dysfunction, despite the thickened pericardial walls. (2) SEM of non-VAD patient pericardium. Normal three layers are visible: mesothelium (white arrow), fibrosa (black arrow) and epipericardium. (3) SEM of VAD patient pericardium. Fibrosa has split into two halves (arrows) that sandwich a newly formed layer of loose tissue (L). Note that the scale in both (2) and (3) is the same. The wavy form of collagen bundles is a mechanically energy-efficient method to deal with the movement that the fibrosa must constantly undergo to follow the rhythm of the cardiac cycle. Similar to an accordion, collagen bundles will extend and contract in diastole and systole, respectively. However, during an episode of sudden and violent tachycardia (common in VAD patients), this rhythm can be greatly increased (up to 200 beats per minute, in a matter of seconds) and the mechanical stress imposed on the MC monolayer may threaten its structural integrity. One of the functions of the loose tissue layer must certainly be blood and nutrient supply to this much larger organ.

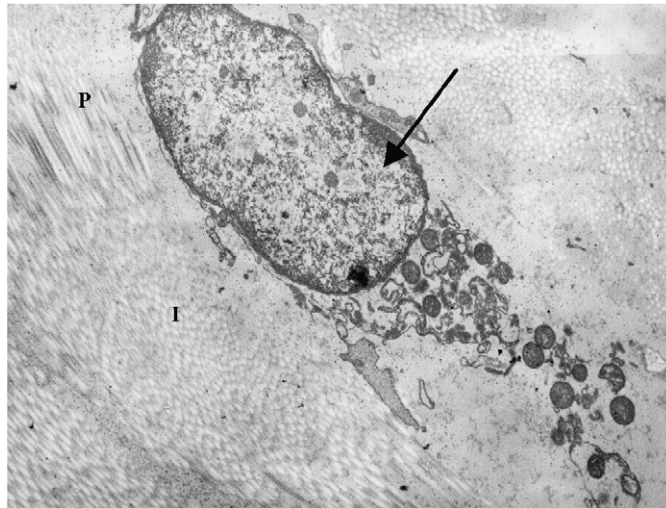


Fig. 3. (TEM) VAD patient parietal pericardium. Deep within the internal fibrosa layer: a burst myofibroblast near a small elastic fibre surrounded by abnormally abundant collagen bundles. Seemingly live organelles are outside the membrane (4000 ×).

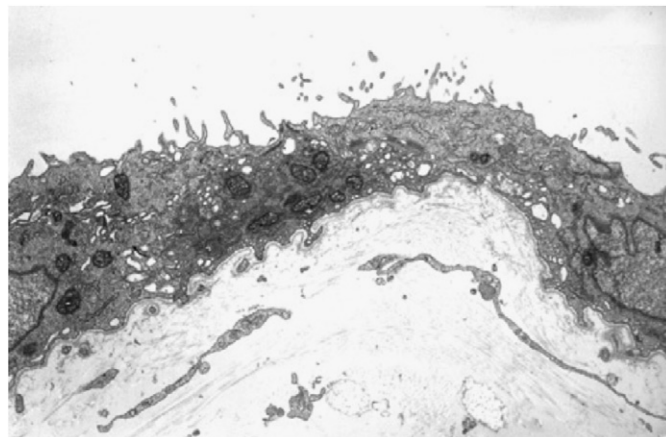


Fig. 4. (TEM) VAD patient pericardial fragment. Pericardial sac (P); Unusual number of cell–cell connections (arrow). Interdigitations (I) linking the MC layer with the subserosal basal layer, through structures reminiscent of anti-seismic constructions (2800 ×).

was seen in all layers and in the vast majority of the images (Fig. 5). Individual, older MC were seen protruding into the pericardial sac, in a process that resembled surface extrusion of that cell (Fig. 6). Discontinuities in MC surface (in direct contact with the pericardial sac) (Fig. 5), with seemingly live organelles spewing into the pericardial sac (Fig. 6) were observed. Desmosomes, which laterally attach adjacent MC (Table 1), were more numerous than would be expected (Fig. 4). The lack of cellular anchoring junctions between MC and the subserosal basal layer (usually accomplished through hemidesmosomes, see Table 1) seems to be replaced by interdigitations whose form is reminiscent of anti-seismic constructions (Fig. 4). MC morphology varied in accordance with the contraction wave of the cardiac rhythm (Castelo Branco et al., 1999b, 2005)

VAD patients often suffer sudden and violent tachycardia, and sudden peaks of increased arterial blood pressure (Castelo Branco, 1999a). This implies a violent and sudden kinetic changes in the cardiac (and pericardial) rhythm. During repeated, VAD-related tachycardia and hypertensive episodes, MC cells become enormously strained, and maintaining structural integrity of the MC monolayer might become an issue. Older

MC cells may have insufficient tensile strength to undergo these violent tachycardia movements, and their extrusion (Fig. 6) from the mesothelium monolayer of cells may be an attempt to maintain structural integrity. The formation of gaps near these older MC cells (Figs. 5 and 6) seems to be an integral part of this extrusion process. The anchoring junctions between the mesothelium and its ECM sublayer seem to have been replaced with MC cytoplasmic interdigitations that dig deep into the ECM sub-layer (Fig. 4). This anchoring structure is not unlike modern anti-seismic constructions, where building blocks fit into each other like pieces in a vertical jigsaw puzzle, providing increased plasticity, and allowing the absorption of large mechanical forces without rupturing.

The existence of such a large amount of cellular debris has been linked to a working hypothesis: The cellular debris seen in VAD patients' pericardial layers can be related to the appearance of auto-immune diseases in ILFN-exposed individuals. Seemingly live organelles that exist outside the cellular membrane envelope (Figs. 3 and 5) will not be identified by the immune system. This could trigger auto-immune disorders. No inflammatory process is tied to the removal of debris. The only visible features are an increased amount of macrophages and neovascularization, with particular relevance to the lymphatic vessels, where the drainage of debris seems to occur.



Fig. 5. (TEM) Pericardial sac (P), collagen bundles (C), cellular debris (x). Discontinuous membrane (arrow), partial loss of cytoplasm. Gaps seen near MC (circle) (4000 $\times$ ).

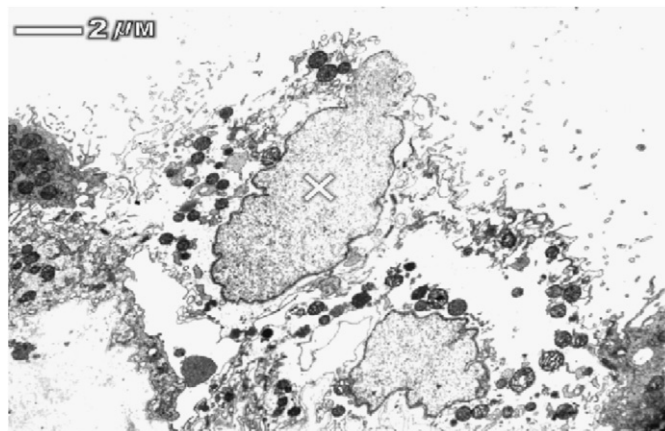


Fig. 6. (TEM) Mesothelial layer with older mesothelial cell (x) in the process of extrusion into the pericardial sac, and with large gaps on either side.

## 5.2. Actin-based structures—brush cell (BC) microvilli and cochlear cilia

BC exist in the respiratory and gastrointestinal tracts. BC possess microvilli uniformly distributed over the apical surface which is known to play a role in increasing absorption surface area. The function and existence of the respiratory BC in humans is largely unknown. In the rat respiratory tract, BC are surrounded by a ring of secretory cells (SC) (Fig. 7) (Castelo Branco et al., 2003e). In ILFN-exposed rodents, microvilli clustered together and, with increasing exposure time, became fused (Fig. 8) (Castelo Branco et al., 2003a, f, g). Cochlear stereocilia also appeared fused in ILFN-exposed rats, both among themselves as well as with the upper tectorial membrane (Figs. 9 and 10) (Alves-Pereira and Castelo Branco, 2003a).

Why BC microvilli respond to prolonged ILFN stress by fusing is unknown. However, the fact that actin filaments can form both rigid (but flexible) bundles as well as gel-like networks, taken together with the fact that motor proteins connect the actin filaments core to the plasma membrane, microvilli fusion does not seem to be such a remote possibility, given the right triggering events.

If fusion of cochlea stereocilia, as a response to prolonged ILFN exposure, also occurs in humans, then this may explain the unusual auditory complaints of VAD patients. Common auditory complaints of VAD patients include “hearing too much” and “not being able to stand any type of noise, not even television or music”. However, their audiograms only present hearing losses within the lower frequency bands (250 and 500 Hz), and their tympanograms are normal (Castelo Branco, 1999a). If fused among themselves and to the tectorial membrane, cilia cannot freely vibrate as is intended when the sound pressure wave is transduced within the cochlea. In fact, by becoming a rigid structure, any attempt at vibrating them might, understandably, produce discomfort. How closely related this phenomenon is to the concept of “annoyance” is still unclear, however a relationship is clearly suggested, especially since annoyance has already been specifically associated with the presence of ILFN (Persson-Waye and Rylander, 2001). In ongoing studies, fusion of actin-based structures in ILFN-exposed rodents has also been observed in the duodenum (Fonseca et al., 2005).

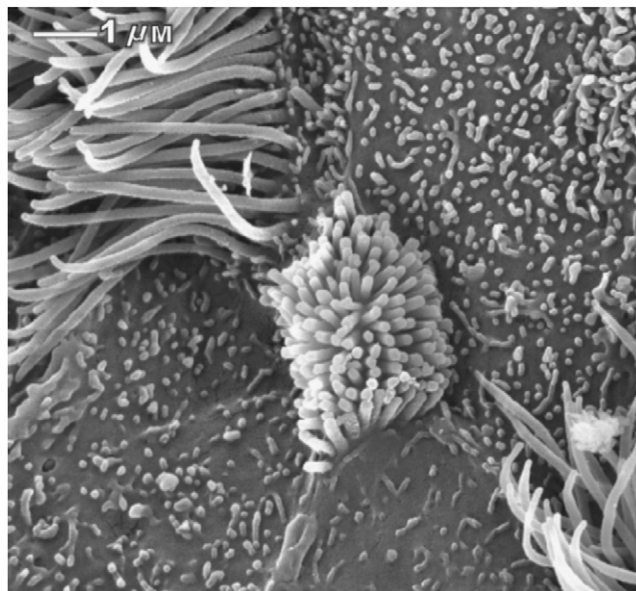


Fig. 7. (SEM) Non-exposed rat bronchial epithelium. The BC, in the center of the image, exhibits a tuft of microvilli that are individually identifiable, uniformly distributed, and sprouting upward into the airway. Surrounding the BC are SC with microvilli of different sizes. Tufts of cilia featuring vesicles are also visible. No sheared, shaggy or wilted cilia are visible. No edema is present.

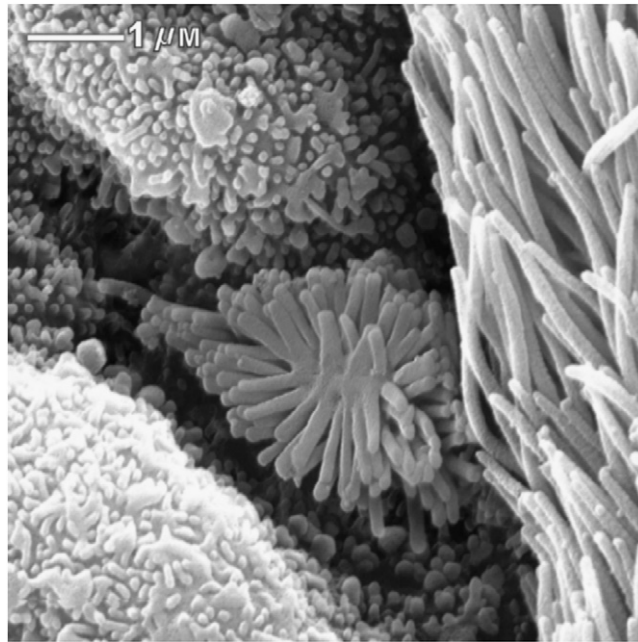


Fig. 8. (SEM) Rat bronchial epithelium exposed to 2160 h of continuous ILFN. A BC is in the centre of the image. Its microvilli are not sprouting upward and, instead, have fused, forming a central indentation that seems to be spreading outward. The prominent SC that surround the BC are swollen forming deep valleys at the intercellular junctions. SC microvilli are very irregular. Cilliary vesicles are visible.

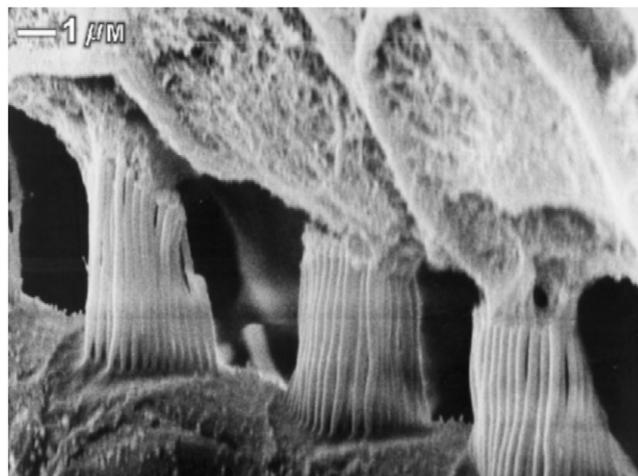


Fig. 9. (SEM) Rat cochlear stereocilia exposed to 4399 h of occupationally simulated ILFN. Cochlear stereocilia are fused amongst themselves and with the upper tectorial membrane.

### 5.3. Other considerations

Given the data obtained to date, the same type of analysis could be made of ILFN-exposed rat kidney glomeruli (Castelo Branco et al., 2003d; Martins dos Santos et al., 2005), or of rat tracheal epithelia (Castelo Branco et al., 2003a, f, g), or of VAD patients bronchoscopic biopsy results (Monteiro et al., 2004; Reis Ferreira et al., 2006), or of VAD patients vocal abnormalities (Mendes et al., 2005, 2006). The behaviour of respiratory tract cilliary populations is of particular interest in that they are composed of tubulin, they are anchored to the CSK actin cortex and, with ILFN exposure, they appear clipped, sheared and/or shaggy

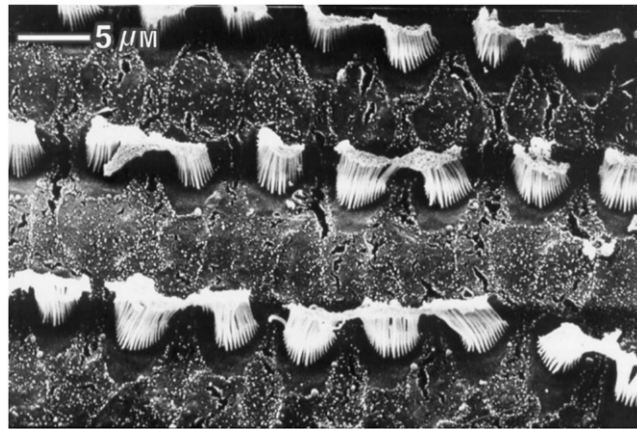


Fig. 10. (SEM) Rat cochlear stereocilia exposed to 4399 h of occupationally simulated ILFN. Cochlear stereocilia after removal of the tectorial membrane, portions of which remain fused to the stereocilia, forming bridges between adjacent cells.

(Alves-Pereira et al., 2003c). Pericardial cilia was also non-existent in VAD patients (Castelo Branco et al., 1999b). The suspicion that the tensegrity model of the cell could explain the findings in ILFN-induced biological structures was hinted at in 1999 (Alves-Pereira) and has since been the object of separate study and independent publications (Alves-Pereira and Castelo Branco, 2003a; Alves-Pereira et al., 2003b–d, 2004c).

Most medical diagnostic procedures are not based on mechanobiological features of disease. Hence, what is analysed, quantified or tested are usually parameters that depend on biochemical pathways. It cannot, therefore, be surprising that VAD present normal routine tests, such as blood chemistry analysis, EKG, and EEG, for example. With echo-imaging, where structural components can be observed, ILFN-induced pathology can be identified. With light and electron microscopy studies, morphofunctional changes can, again, be identified. However, conventional medical tests only become significantly altered in later, and irreversible, stages of VAD (see Table 3). With the tensegrity model of the cell, new avenues of research open up in the area of biochemically based tests from which biomechanical pathology can be extrapolated.

Pharmacological intervention in VAD is several years (and many Euros) away from becoming a reality. However, given that VAD is a mechanotransduction disease *par excellence* it now becomes clear where this intervention must focus: on the cellular signalling processes directly related to mechanotransduction.

## 6. Conclusions

ILFN is neglected as an agent of disease, and mechanotransduction is underestimated as an integral part of cellular signalling. Since VAD is caused by ILFN and explained through mechanotransduction pathways, it is not surprising that it is taking so long for the medical and scientific community to understand its existence. However, with knowledge comes responsibility, and the time has now come to take a more active position against needless suffering. The following recommendations are proposed.

### 6.1. Noise assessment

Hearing impairment is still a major issue within the EU and other countries, thus ceasing to perform measurements with dBA units is not a logical course of action. However, ILFN-rich environments need to be taken into account. Hence, it is proposed that *all* noise measurements be accompanied by a 1/3 octave band analysis, with no weighting (in dBLin), and down to the lowest limiting frequency permitted by the equipment at hand. If spectral analysis is not possible, then at least dBC and/or dBLin Leq measurements should be performed. In this way, not only is the acoustical environment documented for future legal and forensic purposes, but the adequate protection and prevention measures can be taken if it is known how much, and what kind, of ILFN is present in the environment.

The same principle applies to biomedical studies, where ILFN is a possible contaminant. This is particularly true for animal studies where laboratories are kept in building basements, along with HVAC systems and elevator machinery. With human populations, it is important to obtain ILFN-exposure histories, since the effects of ILFN are cumulative (Castelo Branco et al., 1999d) and occur independent of whether the exposure was occupational, residential or leisurely. For example, an office worker may have no immediate exposure to noise on-the-job, but may live next to a bus terminal.

Control populations are especially targeted for comprehensive surveys of previous ILFN-exposure histories because, by definition, they *must not* have prior exposure to ILFN. foetal and leisurely exposures to ILFN must be included in the individual's prior history of ILFN exposure (Castelo Branco et al., 2003a, g; Araújo et al., 2004). The inadequate selection of control populations has already led to wasteful uses of resources and, of course, misleading results (ATSDR, 2001). Given the ubiquitous nature of ILFN, control populations with zero prior ILFN exposure are extremely difficult to gather. Thus, it could be feasible to undertake studies where the bio-effects of ILFN-exposed populations are compared, but only if their acoustical exposures are sufficiently well documented, in terms of exposure times and acoustical spectra.

## 6.2. Dosimetry

Adequate dosimetry of ILFN will be very difficult to achieve until science considers the acoustical spectrum as analogous to the electromagnetic spectrum, i.e., different frequencies have different effects on the different tissues. Thus, breaking the (lower) acoustical spectrum into infrasound versus audible frequencies is much too rudimentary.

It is proposed that the ILFN (0–500 Hz) portion of the acoustical spectrum be divided into the sub-categories listed in Table 4. Biological tissue is very sensitive to lower frequencies, below 100 Hz. Specific frequencies have been known to have a deleterious impact on specific biological tissue (e.g., Nekhoroshev and Glinchikov, 1991, 1992; Svirgovyi and Glinchikov, 1987; Sidorenko et al., 1988), and a 2 Hz exposure can produce different effects than an 8 Hz exposure (Nekhoroshev and Glinchikov, 1991, 1992). Dividing the acoustical spectrum in sub-categories would eventually force bioscientists to specify the acoustical energy within each specific sub-category.

One of the most immediate problems with adequately measuring ILFN is the lack of readily available (and relatively inexpensive) instrumentation. As is well known, much of the monitoring equipment designed to assess physical agents is geared toward assessing the parameters that have been established by legislation. Thus, it is difficult (or much too expensive) to acquire the instrumentation that could adequately measure how long the acoustical energy remains at a certain dB level, for each 1/3 octave band. This would be an ideal parameter for assessing ILFN-induced pathology.

Table 4

Proposed subdivision of the lower portion (0–500 Hz) of acoustical spectrum, by 1/3 octave bands

Sub-category	1/3 octave bands (Hz) <sup>a</sup>	Observations
A	0–6.3	6.3 Hz is often the lower limiting frequency of standard noise measuring equipment software
B	8–12.5	Unusual behaviour in the frequencies of 8, 10 and 12.5 Hz has been detected, in residential, occupational and natural environments (unpublished results)
C	16–25	Overlapping the conventional threshold for human hearing (20 Hz)
D	31.5–63	Where many machines emit noise, includes the 50 Hz associated with high-voltage electrical distribution
E	63–160	Resonance of the thorax
F	200–500	Upper limit, with 250 Hz and 500 Hz already included in audiogram evaluations

<sup>a</sup>The 1/3 octave band analysis divides the acoustical spectrum into frequency bands, referred to by their central frequency. Thus, when measuring in 1/3 octave bands, values are obtained for the 1/3 octave frequency bands whose central frequency is 6.3, 8, 10, 12.5, 16, 20, 25, 31.5, 40, 50, 63, 80, 100, 125, 160, 200, 250, 315, 400 and 500 Hz (see Fig. 1). Hence, in this Table, the apparent discontinuities in the 1/3 Octave Bands column are due to the way in which science segments the acoustical spectrum.

With animal models, some form of ILFN-exposure dosimetry has already been achieved. It is known that after a 48-h continuous exposure to ILFN, it is necessary 7 days for full recovery (Castelo Branco et al., 2003f). However, large-scale epidemiological studies are still lacking, mostly due to the difficulty of selecting adequate control populations, and funding.

### 6.3. Pharmacological intervention

With the integration of the tensigrity cellular model, VAD can now be viewed as a mechanotransduction disease *par excellence*. As such, new avenues of research have opened up regarding the possibility of pharmacological intervention. Since actin- and tubulin-based structures seem to be the most affected, it would make sense to focus on these biomechanical elements in order to avoid irreversible damage to individuals who *must* remain in ILFN environments for extended periods of time, such as ship and submarine workers, offshore oil and gas platforms workers, human activity onboard spacecraft, and the general population who is environmentally exposed to ILFN in the home.

### 6.4. Diagnosing VAD

For the purposes of an informal diagnosis, an echocardiogram to evaluate pericardial and cardiac valve thickening is essential to establish a VAD diagnosis because pericardial thickening with no diastolic dysfunction, and in the absence of an inflammatory process is a specific sign of VAD (Holt, 2000). However, given the limitations of echo-imaging procedures (discussed above) the echocardiogram is insufficient for legal and forensic purposes. Thus, if legal proof of VAD is required, then a more invasive procedure is necessary—the bronchoscopic examination (Reis Ferreira et al., 2006).

Other complementary diagnostic tests include brainstem auditory evoked potentials and cognitive evoked potentials (P300), brain magnetic resonance imaging, PCO<sub>2</sub> rebreathing test, blood coagulation factors and a thorough neurological examination.

Suspicion of VAD should arise if the patient exhibits one or more of the following complaints:

- “I hear too much, I’m very sensitive to noise, I can’t stand any type of noise, Noise drives me crazy, Whenever there’s a loud noise, all I feel like doing is screaming”;
- “I wake up tired, It’s not that I don’t sleep enough hours, it just seems like I don’t rest during my sleep”;
- “Sometimes, while in a shopping mall or a restaurant, I feel like I can’t breath, like I must get out of there or else”;
- “I have a lot of heart palpitations, Sometimes it feels like my heart is going to leap out of my chest”;
- “I have this cough, and I don’t smoke, My throat is constantly irritated and I get hoarse for no reason, The over-the-counter medication doesn’t do anything”.

Or if the patient enters with one of the following diagnosis:

- Late-onset epilepsy;
- Balance disorders;
- Migraine;
- Respiratory tract tumour, especially if a non-smoker;
- Recommendation for cardiac bypass surgery; and
- Auto-immune disease, particularly systemic lupus erythematosus and vitilligo.

The authors urge physicians to listen to their patients and question them about their noise exposures.

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