FILE

17-2295-EL-BGN 29
18-0488-EL-BGN

Dear Ohio Power Siting Board,

I have included a letter I have sent to Professor Christian-Friedrich Vahl in Mainz, Germany, which provides the context for why I am so concerned about the planned wind turbine farms in Seneca County, Ohio. Wind turbine produced infrasound energy, high energy below the audible hearing level, will have adverse health effects on the internal organs of the citizens the Seneca County. Like high blood pressure, it will be a silent cause of organ deterioration over the years. Hundreds of planned wind turbines will have a severe negative health effect on these citizens of Ohio. 21st Century researchers in the European Union today are finding exactly the same harmful effects on internal organs due to infrasound that Soviet researchers found in the 20th Century. I have included research from both areas of Europe, present and past.

The Ohio Government needs to remove infrasound generating wind farms from all renewable energy planning. The choice must be to protect health. Whatever our state plans to do in providing "Green Energy", we must first—do no harm. The state can start by rejecting case project 18-0488-EL-BGN and case project 17-2295-EL-BGN in Seneca and Sandusky Counties.

Sincerely,

Michael T Curran

Michael T. Curran, Captain, USN, (Retired)
Staff, VA Medical Center, Washington D.C., (Retired)

Dear Professor Vahl,

I am writing to you because of the plans by multiple wind power companies and the state government to construct hundreds of wind turbine generators in Seneca County, Ohio in the United States. Because of their plans, I have performed medical literature research at the National Library of Medicine on the campus of the National Institutes of Health in Maryland. From my research, I have read numerous Soviet medical research articles from the 1970's and 1980's which give scathing conclusions on the effect of infrasound on laboratory animal internal organ systems. More recent research by M. Alves-Pereira and N.A.A. Castelo Branco suggest the presence of Vibroacoustic disease. A. Lousinha and her team in Portugal published research in November of this year on the adverse histological development in the cardiac arteries of laboratory rats when exposed to Infrasound.

I have also read the published oral presentation of the research by you and your team in The Thoracic and Cardiovascular Surgeon in February of this year. Also, even though I understand very little of the German language, just today I watched your interview with ZDF German television which I was able to obtain on-line.

I know your research is very recent and possibly still ongoing, but if possible, what I am respectfully requesting is the following:
First, is there, at present, any peer reviewed and formally published articles in any of the medical journals of your team's research that I could use in arguments with state, or local government officials in Ohio against construction of the massive wind turbines planned. (180 meter height)
Second, if your research has not yet been formally published, would there I any way to provide some type of written correspondence from you or your

team which would suggest against the construction of large numbers of wind turbines due to direct negative effects on the contractile ability of heart tissue and the potential long term consequences against human health. I look very forward to hear from you.

Sincerely Yours,

Dr. Michael T. Curran

November 4, 2018 ZDF German Television Interview with Professor Vahl and Documentary on Wind Turbine Infrasound Effects (Dr. Vahl's Laboratory Research also) In German

↓ Go to: ZDF.de → suche —type in→ Vahl → weitere laden ↓ "Infraschall – unerhörter Lärm"

Oral Presentations
Tuesday, February 20, 2018
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Are There Harmful Effects Caused by the Silent Noise of Infrasound Produced by Windparks? An Experimental Approach

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2018 Germany Oral Presentation – ongoing research – Effect: up to 25% reduction in Heart Tissue Contraction Ability

Further Information

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- Congress Abstract
- Full Text

Introduction: The increased number of wind parks raised the question, whether infrasound waves produced by wind turbines are harmful on human-beings, or not. Infrasound is a low frequency sound (< 20 Hz), undetectable with human ears. However, some people live near windparks describe unspecific symptoms i.e., palpitations, dizziness, headache, etc. This study analyses the infrasound effects on isolated atrial human myocardium and measures the contractile performance in human trabeculae using different frequencies and amplitudes of infrasound generated by a loudspeaker.

Methods: Human atrial trabeculae were resected from 8 patients undergoing aorto coronary bypass surgery, then demembranized using Triton X 100 and small fibers were generated with diameter < 0.3 mm and length 4–6 mm. The fibers were attached between force transducer and loudspeaker while activated at optimal length and room temperature in an organ bath using supramaximal calcium concentrations. Then infrasound was imposed using frequencies of 10 Hz or 20 Hz. Sound amplitudes (SA) were either 5% or 10% of tissue length (TL). Sound was applied for 1 minute. Force was measured before and after 1 minute of infrasound.

Results: Imposed infrasound on isolated human myocardium caused a direct force inhibition of the completely activated myocardial preparation. At 10 Hz and 5% TL (SA) force inhibition was 18.8+2% while at 10% TL (SA) up to 23.3+2% ($p < 0.05$). At 20 Hz; force inhibition was 23+2% at 5% TL and 32+4% at 10% TL ($p < 0.01$). After stopping infrasound; force was recovered but not to the initial value. No sound was heard during the experiments. Passive resting force was minimally affected (n.s.).

Conclusion: Infrasound can induce direct effects on human myocardium in the given experimental setting. Although mono-frequency sounds are not present in nature, our experimental data indicates, that direct effects on myocardial tissue are present. The infrasound influence on human tissue requires further investigation because the increasing number of a) wind turbines and b) human beings exposed by the neighborhood of windparks. Humans have no chance to protect themselves from the silent noise of infrasound, as long as no scientific data presents.

EU Research, Portugal
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Original Article

Infrasound induces coronary perivascular fibrosis in rats

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ABSTRACT

Background: Chronic exposure to industrial noise is known to affect biological systems, namely, by inducing fibrosis in the absence of inflammatory cells. In rat hearts exposed to this environmental hazard, we have previously found myocardial and perivascular fibrosis. The acoustic spectrum of industrial environments is particularly rich in high-intensity infrasound (<20 Hz), whose effects on the heart are unknown. We evaluated the morphological changes induced by IFS in rat coronaries in the presence and absence of dexamethasone.
Methods: Adult Wistar rats were divided into three groups: group A (GA)—IFS (<20 Hz, 120 dB)-exposed rats for 28 days treated with dexamethasone; group B (GB)—IFS-exposed rats; group C (GC)—age-matched controls. The midventricle was prepared for observation with an optical microscope using 100× magnification. Thirty-one arterial vessels were selected (GA 8, GB 10, GC 13). The vessel caliber, thickness of the wall, and perivascular dimensions were quantified using *imageJ* software. Mann–Whitney and Kruskal–Wallis tests were used to compare the groups for lumen-to-vessel wall (L/W) and vessel wall-to-perivascular tissue (W/P) ratios.
Results: IFS-exposed rats exhibited a prominent perivascular tissue. The median L/W and median W/P ratios were 0.54 and 0.48, 0.66 and 0.49, and 0.71 and 0.68, respectively, in GA, GB, and GC. The W/P ratio was significantly higher in GC compared with IFS-exposed animals (P=.001). The difference was significant between GC and GB (P=.008) but not between GC and GA.
Conclusion: IFS induces coronary perivascular fibrosis that differs under treatment with corticosteroid.

1. Introduction

Noise represents a major environmental factor and is among the stressors with the highest impact on public health [1]. Noise and sound are physically the same, but the reaction to perception varies between people, depending on the cognitive environment in which detection takes place and ultimately leads to a definition of noise as an undesired sound [2,3]. Low-frequency noise (LFN) and infrasound (IFS) are conventionally defined as sound below 200 and 20 Hz, respectively. The lower limit of the audio frequency range of human hearing is usually given as 16 or 20 Hz, but humans can perceive infrasound if the sound pressure level (dB) is sufficiently high [4]. In the range of IFS, comparative studies have shown that the auditory sensitivity of different species can vary widely. For instance, rats have poorer infrasonic hearing than humans, considering different sound pressure levels [5], but high-intensity (110 dB) IFS vibrations on experimental rats can be perceived, as they elicit active avoidance reactions [6]. Beside its auditory health effects, noise can cause nonauditory effects—such as annoyance, sleep disturbance, and psychological stress—that experimental and epidemiological evidence links to cardiovascular disease, including ischemic heart disease, heart failure, arterial hypertension, arrhythmia, and stroke [7–12].

In recent years, scientists have directed their attention towards the relatively understudied noise range of below 200 Hz. LFN and IFS are present everywhere, from natural occurrences to industrial installations and low-speed machinery. The characteristics of strong penetration and less attenuation in long distance propagation have been proposed to explain several adverse biological effects in experimental and epidemiological studies [13]. Low-frequency sounds have higher energy than the sounds at mid and higher frequencies and cannot be correctly evaluated using the conventional A-filters, which are most often used in environmental studies [14]. It is also possible that there are subtle effects of LFN on the body that we do not yet understand. High sound pressure levels (> = 90 dB) of LFN can induce resonance responses in body cavities [13]. The overall range of human body resonant frequencies was

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1054-8807/

Research references include Soviet era laboratory research with infrasound found to be from 2 to 16 Hz [15], which is nearly the exact range of IFS. It may be assumed that animals also possess inherent specific sound frequencies in certain tissues and organs [16], and for that reason, it is important to document, using animal models, the morphological and biological effects induced by a wide spectrum of wavelengths, from industrial to LFN and IFS.

The cardiovascular system of rodents is sensitive to LFN [17–19]. We previously documented the development of perivascular fibrosis around the coronary arteries (from small to large caliber) of rats exposed to industrial noise [20,21]. We also found a significant fibrotic development in ventricular myocardium among rats submitted to LFN [22,23]. These morphological changes were found in the absence of inflammatory cells, which could suggest a noninflammatory process. However, the fibrotic proliferation mechanism remains unclear.

The effects of IFS on the coronary artery morphology under the influence of an anti-inflammatory agent are unknown. In order to fill this gap, we sought to evaluate the morphological changes induced by IFS in rat coronary arteries in the presence and absence of dexamethasone.

2. Material and methods

Fourteen adult female Wistar rats 10 months old were used in this study. They were purchased from a Spanish breeder (Charles River Laboratories España, S.A., Spain). All the handling and care of the experimental animals were performed by authorized researchers (accredited by the Federation of European Laboratory Animal Science Associations, Category C) and were done in accordance with the EU Commission on Animal Protection for Experimental and Scientific Purposes (2010/63/EU) and with the Portuguese legislation for the same purpose (Decree-Law No. 197/96). The rats were housed in 42×27×16-cm polypropylene cages with a steel lid and had unrestricted access to food (commercial chow) and water. The same standard house conditions were used throughout the experiment for all the animals, and they involved keeping a maximum of two rats in a single cage.

In the beginning of the study, the 14 rats were randomly distributed into three groups. Nine of the rats were continuously exposed to high-intensity and very LFN (2–20 Hz/Lp=114 dB) during a period of 28 days. In four of the noise-treated rats, two tablets of dexamethasone 0.5 mg (Decadron 0.5 mg, Medinfar) were introduced subcutaneously in the dorsal region at two time points of the noise exposure, day one and day 12, and these were designated as group A, while the dexamethasone-free rats were included in group B. The remaining five rats were used as age-matched controls (group C) and sacrificed when all of the rats reached 11 months of age.

2.1. Short description of electroacoustic experiment

With the objective of creating a strong subsonic acoustic field in the *vivarium* chamber, a slightly trapezoidal room with 23.7 m^3 (3.55×3.31×2.02, average length×width×height, respectively, in meters), a pseudo-random waveform in the 2-Hz to 20-Hz decade band was designed with Matlab based on a bandpass-filtered 30-s maximum length sequence segment. The waveform was used to excite an array of two infinite baffles mounted 18-in. 300-W-rated magnetodynamic subwoofers, by means of a 2×600-W heavy-duty quasi-dc voltage output audio power amplifier. Subsequently, with the aim of exploiting as much as possible the available subwoofers dynamic range at this frequency range with an acceptable amplitude distortion, the waveform was iteratively nonlinearly treated with moderate compression-expansion and further filtering (in order to reduce the crest factor to approximately 2.0 times). The total sound pressure level and the spectral characteristics of the resulting acoustic pressure waveform were monitored, and the results were an average sound pressure level of 120 dB with a tolerance of ±3 dB in the 30-s time window. As to the spectral boundedness of the produced sound field, the result was 80 dB total out-of-band average sound pressure level (−40 dB lower).

2.2. Light microscopy

All rats were sacrificed by an intravenous injection of 0.6 ml of a 5:4 mixture containing ketamine (Imalgene 1000, Bayer, Portugal) and xylazine (Rompun, Bayer, Portugal). The vascular system was perfused with a saline solution followed by paraformaldehyde fixation. The heart was excised, sectioned transversely from the ventricular apex to the atria, and routinely processed for light microscopy. The midventricular fragment from each heart was selected for the study. Five-micrometer paraffin-embedded slices of the tissue samples were made and dyed according to Sirius red techniques. The histological images were acquired with an optical microscope using 100× magnification.

2.3. Histomorphometric data

Thirty-one arterial vessels were selected (8 in GA, 10 in GB, and 13 in GC) (Fig. 1). At least one vessel from each rat was included. The researchers, including data collectors and data analysts, were blinded to which group the animals belonged to. Data were analyzed using the *image J* software (National Institutes of Health, Bethesda, MD, USA). The caliber of the arterial vessels, the thickness of the walls, and the perivascular tissue dimension were measured, and for each rat, the mean lumen-to-vessel wall (L/W) and mean vessel wall-to-perivascular tissue (W/P) ratios were calculated (Fig. 2). (See Table 1.)

2.4. Statistical analysis

Mann–Whitney test has been applied in the comparison of IFS-exposed animals (including animals treated with dexamethasone and nontreated animals) and a control group for two parameters: L/W and W/P ratios. Kruskal–Wallis and Mann–Whitney tests were used in the comparison of the three groups for the same parameters. A *P* value <.05 was considered statistically significant.

3. Results

3.1. IFS-exposed animals vs. control animals

The Mann–Whitney test has been used to compare the two groups for L/W ratio and W/P ratio variables, with the Bonferroni correction $\alpha^* = 0.05/2 = 0.025$. The analysis shows that the W/P ratio is significantly lower in the IFS-exposed group (*P*=.001). In contrast, the L/W ratio did not differ between the two groups (*P*=.060). It should be mentioned that the extreme observation for W/P ratio values in the control group does not influence these conclusions, as differences between the groups were still detected by the Mann–Whitney test after removal of that observation (*P*=.003), as expected in view of the robustness of this nonparametric test against such extreme values (Fig. 3).

3.2. Comparison between IFS-exposed dexamethasone-treated animals, IFS-exposed animals, and control animals

In the comparison between the three groups, the Kruskal–Wallis test has been applied with the same Bonferroni correction to the significance level, $\alpha^* = 0.025$. The analysis has shown that there are differences between the groups for W/P ratio (*P*=.011) but not for L/W ratio (P=.104). *Post hoc* comparisons between the groups were conducted for W/P ratio, using the Mann–Whitney test, at the 0.025/3=0.0083 significance level to control for inflation of type 1 error. In this case, differences were detected between control and IFS-exposed animals not treated with dexamethasone (*P*=.008). It should be mentioned that the extreme observation of W/P ratio values does not seem to influence the main conclusion of the Kruskal–Wallis test, as expressed by a significance of .021 of the test result after removal of that observation, but it does change the conclusions of the Mann–Whitney test in the comparison between groups B and C, which is now

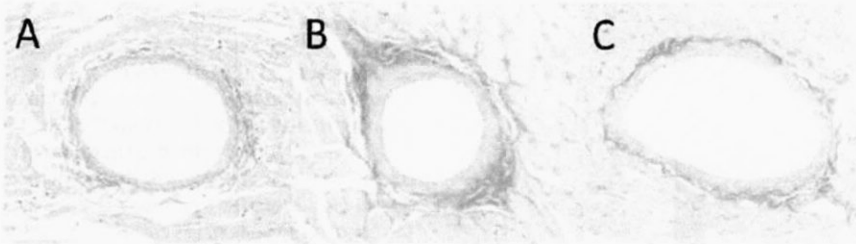

Fig. 1. (A, B, and C) Coronary artery vessels in fragments taken from the left midventricle from (A) group A, infrasound-exposed dexamethasone-treated rats; (B) group B, infrasound-exposed rats; and (C) control group. Note the prominent perivascular tissue in infrasound-exposed animals [Sirius red, 100×].

nonsignificant (P=.021) under the Bonferroni correction (* = 0.0083) (Fig. 4).

4. Discussion

The present study evaluated the coronary morphological changes in rat heart induced by pure IFS, created in a laboratory controlled electroacoustic experiment, and is the first study assessing the possible influence of an anti-inflammatory agent on these changes.

In this investigation, we found an increase in the perivascular tissue around the coronaries in rats exposed to IFS. There were significant differences between IFS-exposed rats and controls concerning the mean W/P ratio, higher among the control group (P<.001). But such differences did not reach statistical significance in the comparison between the animals treated with dexamethasone and the control group, pointing to a possible influence of this potent anti-inflammatory agent.

Previous work from our group, in Wistar rats, investigated the histomorphometric changes in the large and small coronary arteries induced by high-intensity industrial noise within a wide spectrum of wavelengths that included LFN, this last characterized by large sound pressure amplitude ≥90 dB and low-frequency bands of ≤500 Hz [20, 21]. The exposure time ranged from 1 to 7 months. In both studies, we found the development of perivascular fibrosis in the absence of inflammatory cells, regardless of exposure time. In another study, we have documented a significant fibrotic development in ventricular myocardium of rats exposed to LFN during a period of 3 months [22]. These investigations confirmed the abnormal proliferation of connective tissue as the main morphological change induced by LFN.

With increasing urbanization, noise is rising as one of the most important environmental risk factors in modern societies. The importance of the characteristics of the noise stimulus, such as frequency content, intensity, mean and peak dB level, pattern, and exposure time, is not well understood. In the quantitative risk assessment of environmental noise, the World Health Organization (WHO) Regional Office for Europe is concerned with sound pressure level limits, not frequencies [1]. Nonetheless, WHO also acknowledges the special place of LFN as an environmental problem, recognizing that the evidence is sufficiently strong to warrant immediate concern.

Sources of LFN include natural occurrences, industrial installations, and low-speed machinery, ranging from very low-frequency atmospheric fluctuations up to lower audio frequencies. Due to the characteristics of strong penetration and less attenuation in long distance propagation, it has been implicated in several adverse biological effects in experimental and epidemiological studies [13].

One effect of high pressure levels of LFN is excitation of body vibrations [13,19,24]. At high sound levels, typically above 80 dB, the occurrence of resonance responses in body cavities was described [24]. The overall range of human body resonant frequencies was found to be from 2 to 16 Hz [15], which is almost the exact range of infrasound. The displacement between the organ and the skeletal structure places biodynamic strain on the body tissue involved, and it is known to reach its maximum under exposure to vibration close to the body's resonant frequency. Despite the practical impossibility of stimulating the natural frequency of one organ alone without exciting the whole-body resonances, measurements of vibration transmissibility from the point of excitation to a specific organ reveal frequencies of maximum transmissibility that can be attributed to the resonance of the organ. Considering that animals also possess inherent specific sound frequencies in certain tissues and organs [16], it is important to assess the morphological and biological effects induced by noise with different wavelengths in distinct animal models. So far, we have focused our investigation on the effects of large pressure amplitude noise within a wide spectrum of wavelengths, from the industrial to LFN and IFS, and with different exposure times, from 1 to several months [20–23]. The common finding was an abnormal deposition of collagen in the extracellular matrix (ECM), regardless of the characteristics of the noise stimulus other than pressure amplitude.

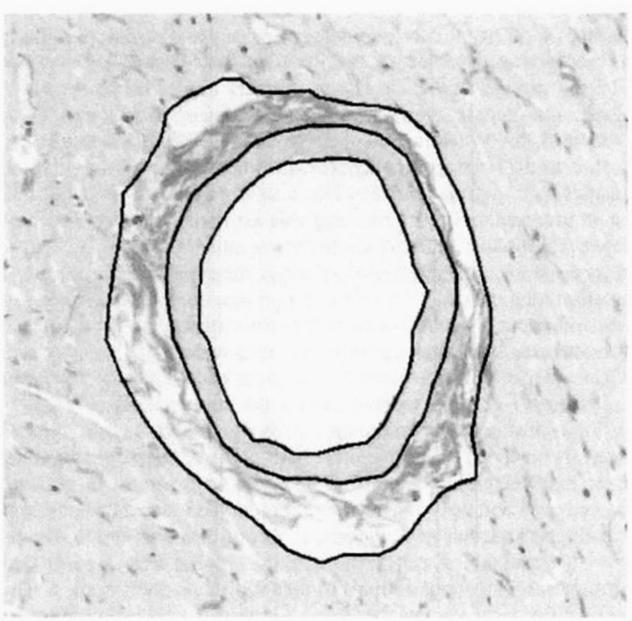

Fig. 2. Example of a coronary artery in a fragment taken from the left midventricle of an infrasound exposed rat [Sirius red, 100×]. The black lines represent the measurements performed using *Image j* software and correspond to vessel caliber, thickness of the wall, and perivascular dimension. These were used to calculate the L/W and W/P ratios.

Table 1
Median (interquartile range) of the two measured outcomes in the three groups

	Ratio L/W Median (interquartile range)	Ratio W/P Median (interquartile range)
Group A	0.54 (0.17)	0.48 (0.15)
Group B	0.66 (0.09)	0.49 (0.08)
Group C	0.71 (0.10)	0.68 (0.08)

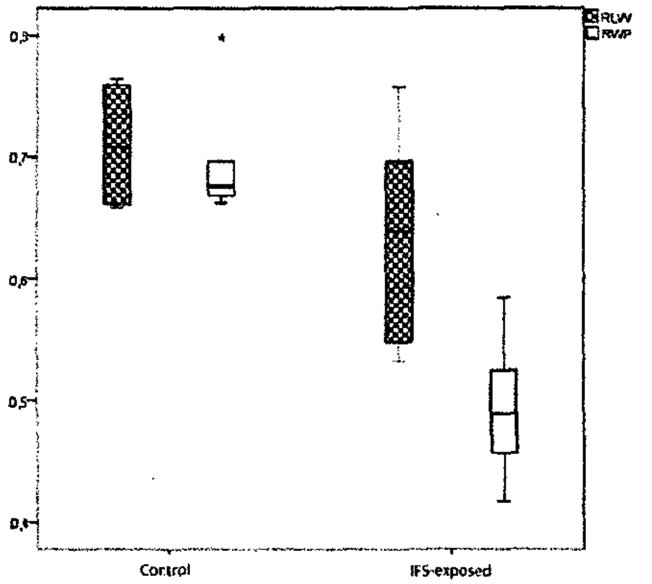

Fig. 3. Lumen-to-vessel wall and vessel wall-to-perivascular tissue ratios in IFS-exposed and control animals. The W/P ratio was significantly reduced in IFS-exposed animals (P=.001). RLW, lumen-to-vessel wall ratio; RWP, vessel wall-to-perivascular tissue ratio.

Interest in the potential adverse health effects of IFS has increased over time. High-level IFS below 20 Hz was historically thought to be of much less significance than LFN in the 20–200 Hz range at the same pressure level [25]. Research on the impact of IFS on the environment established that, for levels above 120 dB, it is dangerous to the human body [13].

Infrasound exposure studies in laboratory animals are scarce and report adverse effects in the ear and auditory system [26], brain and central nervous system [27,28], liver [29,30], and lung [31]. Specifically, the cardiovascular system is sensitive to IFS, as shown by the first studies conducted more than 25 years ago. In these studies, rats were exposed to infrasound (4, 8, and/or 16 Hz at 90 to 145 dB) for up to 45 days, which ultimately led to myocardial ischemia and morphofunctional changes in the myocardium cells [32–34]. More recently, Pei et al. reported IFS-induced hemodynamics, cardiac ultrastructure damage, and cardiac cell apoptosis in the rat myocardium [35,36]. The same group found that IFS dysregulates the L-type calcium currents in rat ventricular myocytes [16] and also that acute exposure to IFS induces oxidative damage of cardiomyocytes that affects a series of oxidative damage-related proteins and genes, suggesting a complex signaling network that is evoked by this stressor [37].

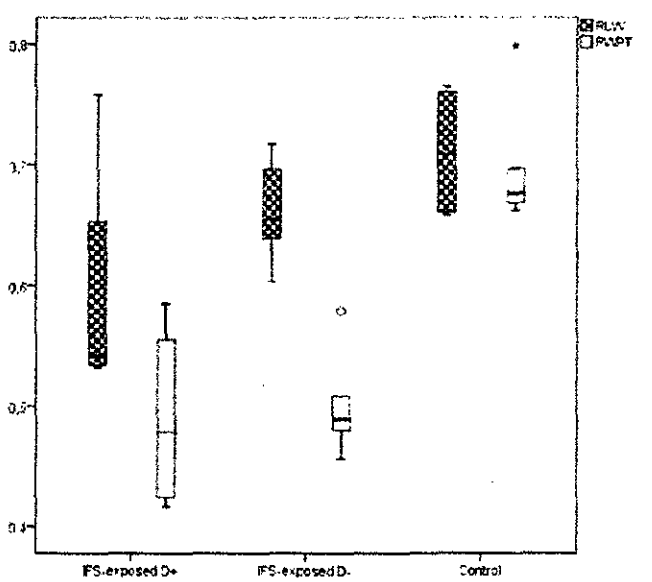

Fig. 4. Lumen-to-vessel wall and vessel wall-to-perivascular tissue ratios in infrasound-exposed dexamethasone-treated rats (group A), infrasound-exposed rats (group B), and control group (group C). For W/P ratio, there are differences between the groups (P=.011) and between groups B and C (P=.008), but not between groups A and C. RLW, lumen-to-vessel wall ratio; RWP, vessel wall-to-perivascular tissue ratio. D+ and D−, dexamethasone-treated and not treated, respectively.

There is no agreement about the biological activity of LFN and IFS and the possible underlying mechanisms. The biological effects of noise on living bodies may not be the same due to different parameters such as biological species, frequency, level of sound pressure, or time of exposure. Over the last years, an increased focus from investigators towards the elucidation of these questions has been observed. Increased release of stress hormones, activation of sympathetic nervous system, increased reactive oxygen species production, endothelial dysfunction, peripheral vasoconstriction, increased peripheral vascular resistance, and increased blood viscosity are among the proposed mechanisms elicited by acute or chronic noise stress leading to detrimental outcomes on the cardiovascular system [7,9,38]. Following this line of investigation, Said and El-Gohary studied the effect of noise in the 80–100-dB range on heart rate and mean systemic arterial blood pressure in adult male albino rats and explored possible underlying mechanisms [39]. They concluded that noise stress has many adverse effects on cardiovascular system through increasing plasma levels of stress hormones, oxidative stress, and endothelial dysfunction.

Until recently, it was presumed that LFN required greater sound pressure in order to elicit toxicological effects on humans and animals. High sound pressure levels can be harmful to the cochlea and cause hearing loss, raising the question of other noise effects being secondary, at least partially, to direct auditory damage. Since animal models in previous studies employed mainly high dBA levels (>100–120 dBA), some investigators started exploring the effects of low decibel noise. Jin et al. [17] used isolated and cultured cardiac fibroblasts from rats to study the effects of low decibel IFS. They reported that noise below 90 dB at 4–20 Hz inhibits angiotensin-II-stimulated cardiac fibroblasts by reactivating miR-29a targeting the TGF- /Smad3 pathway, possibly eliciting cardiac protective effects. Munzel et al. [18] developed a novel noise exposure model in mice with lower peak sound levels (<85 dBA), lower mean sound pressure levels (72 dBA), and shorter exposure times (1–4 days), thought to cause mainly nonauditory effects to animals such as stress reactions. Exposure to noise resulted in elevated blood pressure and heart rate and was associated with detrimental changes in vascular endothelial function, vascular production of reactive oxygen species, and increased blood stress hormones and biomarkers of inflammation. Notably, they describe an invasion of the vasculature with inflammatory cells. The same group demonstrated that nighttime aircraft noise in healthy volunteers causes endothelial dysfunction, which was partially corrected by the acute administration of vitamin C, pointing to increased oxidative stress as a key mechanism [40].

There are currently limited data on the hypothetical noise-induced pathway involving inflammation [11]. In humans, sleep disturbance is associated with a proinflammatory state [41]. As previously mentioned, the common finding in the noise experiments conducted by our group was the perivascular and myocardial fibrotic development in the absence of inflammatory cells [20–23]. In the present study, we included a group of IFS-exposed animals treated with dexamethasone, a synthetic glucocorticoid member with immunosuppressive potency of about 20–30 times that of hydrocortisone and 4–5 times of prednisone [42,43]. Subcutaneous application of dexamethasone, in contrast to intraperitoneal, is highly effective in inhibiting inflammation in mouse models even at low doses [44]. Interestingly, we found differences in the comparison of control group with IFS-exposed animals with and without dexamethasone treatment, as the treated animals did not show significant differences when compared to controls. This is the first time that such differences are documented, and despite the absence of inflammatory cells previously described by our group, we have to consider a potential underlying inflammatory mechanism.

The mechanism behind the fibrotic proliferation induced by noise in rat heart is not yet understood. In general, the differentiation of cardiac fibroblasts into more active myofibroblasts is the hallmark of cardiac fibrosis, leading to an abnormal accumulation of the ECM components, such as collagen, around damaged heart tissues [45,46].

Myofibroblast differentiation is a complex and highly regulated process, where biochemical and mechanical factors are interdependent [47]. From a biochemical aspect, the differentiation of cardiac fibroblasts into myofibroblasts is well studied, while the role of mechanical factors remains elusive [48]. When exposed to abnormal mechanical conditions such as strain and ECM stiffness, cardiac fibroblasts can undergo myofibroblast differentiation [49,50]. A fact worth mentioning within the scope of our investigation is that, during the cellular response to heart injury, myofibroblasts actively secrete ECM proteins, such as collagen I and III, to replace the damaged myocardium [51]. We previously performed an immunohistochemical and electron microscopy study in order to evaluate the effects of LFN on cardiac collagen and cardiomyocyte ultrastructure [23]. A significant increase of collagens I and III in the ECM was observed. The ultrastructural observation denoted high concentration of collagen in the ECM next to fibroblasts, confirming the pronounced effect of LFN on the connective tissue.

Comparable to the traditional cardiovascular risk factors, experimental and epidemiological evidence substantiates the concept that noise, through auditory and nonauditory effects, may induce activation of different pathways (oxidative stress, vascular dysfunction, autonomic imbalance) that ultimately lead to cardiac fibrosis, adverse ventricular remodeling, and arrhythmogenesis [7–12]. It is important to note that nonauditory noise effects (annoyance, sleep disturbance, and psychological stress) do not follow the toxicological principle of dosage [7]. Consequently, not simply the accumulated sound energy that causes the adverse effect but also the cognitive perception of the sound, the subsequent cortical activation, and the emotional response need to be taken into account. More epidemiological research on LFN and health effects is needed since the available research is scarce and suffers from methodological shortcomings. A systematic review of observational studies suggests an association between everyday life LFN and IFS components (up to 250 Hz) and health effects in the general population, such as annoyance, sleep-related problems, concentration difficulties, and headache [52]. However, they underline the inconsistency across studies and the small number of existing observational investigations, precluding a direct comparison with experimental evidence.

This study has some limitations. The number of animals per group was limited; therefore, the results should be interpreted cautiously. The significant correlation between the two dependent variables considered in this study, ratio L/W and ratio W/P, as expressed by a Spearman correlation coefficient of 0.705 (P=.005), would recommend a multivariate approach to the data in order to account for the effect of the association between variables on type I error. However, given the reduced dimensions of the groups, it is not recommended to assess the multivariate normality and homogeneity of variance–covariance assumptions in view of the reduced power of the corresponding tests. In these conditions, the Mann–Whitney test has been used to compare the two groups for ratio L/W and ratio W/P variables, with the Bonferroni correction $^* = 0.05/2 = 0.025$. For the reasons mentioned above regarding the correlation between the dependent variables and group dimension, a nonparametric approach to the data was implemented in the comparison between three groups. The Kruskal–Wallis test has been applied with the same Bonferroni correction to the significance level, $^* = 0.025$, and post hoc comparisons between the groups were conducted for ratio W/P using the Mann–Whitney test, at the $0.025/3 = 0.0083$ significance level, to control for inflation of type 1 error. Also, experimental noise stress models are scarce, and at the present time, a well-defined morphological cardiac model to study the consequences of IFS exposure does not exist. There is a lack of consensus regarding the cardiac cell composition, including fibroblasts, in mammals, with potential variations between species that also depend on the age [53]. Concerning the characteristics of noise, public health research uses A-weighting method to measure noise and focus on sound pressure level, disregarding frequencies. We believe that both sound frequency and intensity are key factors. So far, we investigated the structural modifications in the rat myocardium induced by high sound pressure noise of different wavelengths, from industrial to IFS. Addressing these important questions at the mechanistic level in animals may help provide directions for studies in humans, as more epidemiological research is imperative.

5. Conclusions

Infrasound exposure induces coronary perivascular fibrosis that differs under corticosteroid administration, which raises the possibility of an underlying inflammatory mechanism. The importance of noise in perturbation of inflammatory factors needs to be further investigated.

Con icts of interest

None.

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References

[1] Fritschi L, Brown AL, Kim R, Schwela DH, Kephalopoulos S. Burden of disease from environmental noise. World Health Organization; 2011http://www.euro.who.int/_data/assets/pdf_file/0008/136466/e94888.pdf.
[2] Guski R. Personal and social variables as co-determinants of noise annoyance. Noise Health 1999;1:45–56.
[3] Belojevic G, Jakovljevic B. Factors influencing subjective noise sensitivity in an urban population. Noise Health 2001;4:17–24.
[4] Moller H, Pedersen CS. Hearing at low and infrasonic frequencies. Noise Health 2004;6:37–57.
[5] Heffner HE, Heffner RS. Hearing ranges of laboratory animals. J Am Assoc Lab Anim Sci 2007;46(1):20–2.
[6] Berezhnoy DS, Kiselev NA, Novoseletskaya NM, Inozemtsev AN. Influence of high-intensity sound vibration on rat behavior. Moscow Univ Biol Sci Bull 2015;70(2):53–7.
[7] Babisch W. Cardiovascular effects of noise. Noise Health 2011;13:201–4.
[8] Basner M, Babisch W, Davis A, et al. Auditory and non-auditory effects of noise on health. Lancet 2014;383:1325–32.
[9] Munzel T, Gori T, Babisch W, Basner M. Cardiovascular effects of environmental noise exposure. Eur Heart J 2014;35:829–36.
[10] Munzel T, Sorensen M, Gori T, et al. Environmental stressors and cardio-metabolic disease: part I—epidemiologic evidence supporting a role for noise and air pollution and effects of mitigation strategies. Eur Heart J 2017;38:550–6.
[11] Munzel T, Sorensen M, Gori T, et al. Environmental stressors and cardio-metabolic disease: part II—mechanistic insights. Eur Heart J 2017;38:557–64.
[12] Cai Y, Hansell AL, Blangiardo M, et al. Long-term exposure to road traffic noise, ambient air pollution, and cardiovascular risk factors in the HUNT and lifeline cohorts. Eur Heart J 2017;38:2290–6.
[13] Leventhall G. What is infrasound? Prog Biophys Mol Biol 2007;93:130–7.
[14] Ziaran S. The assessment and evaluation of low-frequency noise near the region of infrasound. Noise Health 2014;16:10–7.
[15] Randall JM, Matthews RT, Stiles MA. Resonant frequencies of standing humans. Ergonomics 1997;40:879–86.
[16] Pei Z, Zhuang Z, Xiao P, et al. Influence of infrasound exposure on the whole L-type calcium currents in rat ventricular myocytes. Cardiovasc Toxicol 2009;9:70–7.
[17] Jin W, Deng Q, Chen B, et al. Inhibitory effects of low decibel infrasound on the cardiac fibroblasts and the involved mechanism. Noise Health 2017;19:149–53.
[18] Munzel T, Daiber A, Steven S, et al. Effects of noise on vascular function, oxidative stress, and inflammation: mechanistic insight from studies in mice. Eur Heart J 2017;38:2838–49.
[19] Wu CC, Chen SJ, Yen MH. Effects of noise on blood pressure and vascular reactivities. Clin Exp Pharmacol Physiol 1992;19:833–8.

[20] Lousinha A, Antunes E, Borrecho G, Oliveira MJ, Brito J, Martins dos Santos J. Histomorphometric evaluation of the small coronary arteries in rats exposed to industrial noise. Int J Mol Sci 2015;16:1095–104.
[21] Antunes E, Oliveira P, Oliveira MJ, Brito J, Águas A, Martins dos Santos J. Histomorphometric evaluation of the coronary artery vessels in rats submitted to industrial noise. Acta Cardiol 2013;68:241–5.
[22] Antunes E, Oliveira P, Borrecho G, et al. Myocardial fibrosis in rats exposed to low-frequency noise. Acta Cardiol 2013;68:285–9.
[23] Antunes E, Borrecho G, Oliveira P, et al. Effects of low-frequency noise on cardiac collagen and cardiomyocyte ultrastructure: an immunohistochemical and electron microscopy study. Int J Clin Exp Pathol 2013;6:2333–41.
[24] Smith SD. Characterizing the effects of airborne vibration on human body vibration response. Aviat Space Environ Med 2002;73:36–45.
[25] Broner N. The effects of low frequency noise on people—a review. J Sound Vib 1978; 58:483–500.
[26] Bohne BA, Harding GW. Degeneration in the cochlea after noise damage. Primary versus secondary events. Am J Otol 2000;21:505–9.
[27] Nekhoroshev AS, Glinchikov VV. Effects of infrasound on change in the auditory cortex. Gig Sanit 1992:62–4.
[28] Liu J, Lin T, Yan X, et al. Effects of infrasound on cell proliferation in the dentate gyrus of adult rats. Neuroreport 2010;21:585–9.
[29] Alekseev SV, Glinchikov VV, Usenko VR. Reaction of the liver cells to infrasound. Noise Vib Bull 1987:131–2.
[30] Nekhoroshev AS, Glinchikov VV. Morphological research on the liver structures of experimental animals under the action of infrasound. Aviakosm Ekolog Med 1992;26:56–9.
[31] Svidovyi VI, Glinchikov VV. Action of infrasound on the lung structure. Noise Vib Bull 1987:153–4.
[32] Alekseev SV, Glinchikov VV, Usenko VR. Myocardial ischemia in rats exposed to infrasound. Gig Tr Prof Zabol 1983;8:34–8.
[33] Gordeladze AS, Glinchikov VV, Usenko VR. Experimental myocardial ischemia caused by infrasound. Gig Tr Prof Zabol 1986;6:30–3.
[34] Nekhoroshev AS, Glinchikov VV. Morpho-functional changes in the myocardium after exposure to infrasound. Gig Sanit 1991:12:56–8.
[35] Pei Z, Sang H, Li R, et al. Infrasound-induced hemodynamics, ultrastructure, and molecular changes in the rat myocardium. Environ Toxicol 2007;22:169–75.
[36] Pei ZH, Chen BY, Tie R, et al. Infrasound exposure induces apoptosis of rat cardiac myocytes by regulating the expression of apoptosis-related proteins. Cardiovasc Toxicol 2011;11:341–6.
[37] Pei Z, Meng R, Zhuang Z, et al. Cardiac peroxisome proliferator-activated receptor-γ expression is modulated by oxidative stress in acutely infrasound-exposed cardiomyocytes. Cardiovasc Toxicol 2013;13:307–15.
[38] Baldwin AL, Wagers C, Schwartz GE. Reiki improves heart rate homeostasis in laboratory rats. J Altern Complement Med 2008;14:417–22.
[39] Said M, El-Gohary O. Effect of noise stress on cardiovascular system in adult male albino rat: implication of stress hormone, endothelial dysfunction and oxidative stress. Gen Physiol Biophys 2016;35:371–7.
[40] Schmidt FP, Basner M, Kroger G, et al. Effect of nighttime aircraft noise exposure on endothelial function and stress hormone release in healthy adults. Eur Heart J 2013; 34:3508–14.
[41] Irwin MR, Olmstead R, Carrol JE. Sleep disturbance, sleep duration, and inflammation: a systematic review and meta-analysis of cohort studies and experimental sleep deprivation. Biol Psychiatry 2016;80:40–52.
[42] Rhen T, Cidlowski JA. Anti-inflammatory action of glucocorticoids—new mechanisms for old drugs. N Engl J Med 2005;353:1711–23.
[43] Mager DE, Moledina N, Jusko WJ. Relative immunosuppressive potency of therapeutic corticosteroids measured by whole blood lymphocyte proliferation. J Pharm Sci 2003;92:1521–5.
[44] Weichhart T, Brandt O, Lassnig C, et al. The anti-inflammatory potency of dexamethasone is determined by the route of application in vivo. Immunol Lett 2010;129: 50–2.
[45] Borne SW, Diez J, Blankesteijn WM, Verjans J, Hofstra L, Narula J. Myocardial remodeling after infarction: the role of myofibroblasts. Nat Rev Cardiol 2010;7:30–7.
[46] Wynn TA, Ramalingam TR. Mechanisms of fibrosis: therapeutic translation for fibrotic disease. Nat Med 2012;18:1028–40.
[47] MacKenna D, Summerour SR, Villarreal FJ. Role of mechanical factors in modulating cardiac fibroblast function and extracellular matrix synthesis. Cardiovasc Res 2000; 46;257–63.
[48] Yong KW, Li Y, Huang G, et al. Mechanoregulation of cardiac myofibroblast differentiation: implications for cardiac fibrosis and therapy. Am J Physiol Heart Circ Physiol 2015;309:H532–42.
[49] Costa AP, Clemente CF, Carvalho HF, Carvalheira JB, Nadruz W, Franchini KG. FAK mediates the activation of cardiac fibroblasts induced by mechanical stress through regulation of the mTOR complex. Cardiovasc Res 2010;86:421–31.
[50] Hinz B. The myofibroblast: paradigm for a mechanically active cell. J Biomech 2010; 43:146–55.
[51] Ma Y, Bras LE, Toba H, et al. Myofibroblasts and the extracellular matrix network in post-myocardial infarction cardiac remodeling. Pflügers Arch 2014;466:1113–27.
[52] Baliatsas C, van Kamp I, van Poll R, Yzermans J. Health effects from low-frequency noise and infrasound in the general population: is it time to listen? A systematic review of observational studies. Sci Total Environ 2016;557-8:163–9.
[53] Pinto AR, Ilinykh A, Ivey MJ, et al. Revisiting cardiac cell composition. Circ Res 2016; 118:400–9.

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EXPERIMENTAL MYOCARDIAL ISCHEMIA CAUSED BY INFRASOUND

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Modern scientific and technological progress has led to the creation of large-sized machines and units capable to generate infrasonic vibrations, which are an integral part of production noise. At certain parameters, infrasound can have a harmful effect on the body [4]. Currently, an intensive study of the effect of infrasound over both the whole organism, and on various organs and tissues is being conducted [3).

There are works in the literature showing the effect of infrasound over the myocardium [1, 2, 5, 6). These studies stated that infrasound damages firstly the vessels of the myocardium. At the same time, the pathogenesis of the effect of infrasound over the myocardium remains unclear in many details, the effect of infrasound over the structures of cardiomyocytes has not been studied, which is the goal of the present study.

For holding experiments, a specially constructed acoustic installation was used, allowing to create the infrasonic field in the range of 0.5–50 Hz with a pressure intensity of 90–140 dB.

The experiments were performed on white rats and guinea pigs, which were irradiated with infrasound at a frequency of 8 Hz, intensity of 120 dB during 1, 5, 10, 15, 25, 40 days with daily exposure of 3 hours. In all groups of experiments 10 animals were used, 3 of which served as a control. Animals were slaughtered by decapitation. Fixation was performed by Carnoy method and in 20% formalin.

The preparations were stained with hematoxylin-eosin, by Van Gieson method, halo-cyanin, by Einarson method, to detect nucleic acids, methyl green with pyronin, by Brache method (control with ribonuclease). The SCHIFF- reaction with amylase control was used, the activity of succinate dehydrogenase (LDH), lactate dehydrogenase (LDH), and glucose-6-phosphate dehydrogenase (G-6-FDG) were also investigated. To detect the activity of redox enzymes, cooled myocardium was cut in the cryostat at a temperature of —5 ° C. Cuttings were processed according to E. Pierce's prescriptions. Evaluation of histochemical reactions and the activity of redox enzymes was performed by a semi-quantitative method, comparing the obtained data with the control.

For electron microscopic examination, fixation was performed with 2.5% glutaraldehyde for 2 hours with additional fixation with 1% osmium and with subsequent dehydration with alcohol. Ultrathin cuttings were made on the LKB-III ultratome, contrasted with lead citrate and examined with electron microscope JEM-7A.

In acute experiments after 3-hour single. exposure to infrasound with a frequency of 7 Hz and an intensity of 120 dV, when examining the heart, barely perceptible pallor and swelling of the left and right ventricular walls and small-point hemorrhage in the pericardium structure were noted.

Histological examination showed mild edema and in some cardiomyocytes - moderate grit and even vacuolar dystrophy of myofibrils with the disappearance of ransverse striation. SCHIFF -reaction was unevenly expressed, weakened after treatment of cuttings with amylase. Pironnofilia was of diffuse nature and decreased after exposure to nuclease. The activity of the LDH was increased, the precipitated grains of diformazan differed in polymorphism. Capillary lumens are filled with red blood cells, but endothelial cells look swollen.

During electron microscopic examination, reactively altered cardiomyocytes show mitochondrial swelling and destruction of outer membranes with loss of dual contour, enlightenment and homogenization of the matrix with fragmentation of the cristae. In myofibrils there are areas of re-coloring, and sometimes tears of myofilaments in the area of the disks. The canals of the T-system are dilated. An increase in the amount of chromatin is noted in the nuclei. Nuclear pores are enlarged.

With continued impact of infrasound, a day after the start of the experiment, the activity of redox enzymes falls in the ischemic zone, but at the same time there are areas in which myofibrils are painted over with aggregation of diformazan grains. Reactively modified cardiomyocytes give a weak SCHIFF -positive reaction, weakening when treating medicines with amylase. Pironnofilia has a diffuse character. The activity of SDH varies, at first decreasing sharply compared to the control one, then increasing. The activity of LDH in some myofibrils is increased. The activity of G-6-FDG and NAD-diaphorase is expressed weak. In the foci of ischemia, the capillaries are sharply narrowed as a result of the swelling of the endothelium cells. The sarcoplasm of cardiomyocytes is edematous, the sarcolemma is damaged in a number of areas, there are homogenization zones and a re-dyeing band in the myofibrils. The mitochondria are swollen, with a vague outer membrane, devoid of matrix, the cristae are fragmented to varying degrees. The contours of the nuclei are strengthened, the nucleoli disappear, the amount of chromatin is increased, the nuclear pores are enlarged. In the T-system there are vacuoles of various sizes, the sarcoplasmic reticulum canaliculi are enlarged.

In the intact zones, single modified cardiomyocytes appear with the presence of re-dyeing bands and even with damage of myofilaments.

At the 5th, 10th, and 15th day, in the zones of myocardial ischemia located mosaically in the region of the left ventricle, there are perivaecular hemorrhages around the small vessels, and there are separate leukocytes in the surrounding connective tissue. Damaged infrasound cardiomyomatics changed, they have all the signs of granular dystrophy. The SCHIFF reaction is poorly expressed, does not change after treatment with amylase, pyronnophilia is focal in nature and disappears after treatment with ribonuclease. The a/ctivity of redox enzymes is reduced, the myofibrils are diffusely stained, the diformazan grains form polymorphic clusters. Sarcoplasma of cardiomyocytes is edematous, in some places myofibrils are fragmented in the area of the discs, there are foci of homogenization of myofilaments (see figure); discs are mixed and expanded. Many mitochondria are swollen, with a spotty-coated matrix, the cristae are finely fragmented, the outer membrane in a number of structures is devoid of dual contour. The contours of the nuclei are deformed, nucleoplasm is cleared in some places, chromatin forms clusters of irregular shape. Sarco-plasma reticulum canaliculi dilated. The erythrocytes accumulate in the lumens of the dilated capillaries, and in the swollen endothelial cells there are destroyed mitochondria

After 25 and 40 days of infrasound impact in the area of myocardial ischemia, the SCHIC-reaction of cardiomyocytes is weak. Pironnofilia of cells has a focal character and decreases after treatment with ribonuclease. The activity of redox enzymes increases, there are areas with myofibrils stained in color, the diformazan grains form focal accumulations. The activity of G-6-FDG increases.

At the 25th day in reactively altered cardiomyocytes, sarcoplasm edema decreases. Sarcolemma is sharply contoured, the number of ribosomes increases, however, myofilaments are homogenized in some places. Mitochondria have an oval shape, in the matrix there are sometimes foci of enlightenment, the crista are in most cases parallel to each other, fragmentation is poorly noticeable. The nuclei of cells have rugged but clear contours, chromatin is located in the form of clumps of various sizes, the pores of the nuclear membrane are enlarged. The lamellar complex is little changed, the tubules of the sarco-plasma reticulum and the T-system are moderately dilated. There are single lipid inclusions, sometimes primary and secondary lysosomes are found. Capillary openings are enlarged, the amount of chromatin in the nuclei is increased, the mitochondria are homogenized, the number of glycogen granules is reduced.

Full restoration of damaged cardiac cells occurs as a result of intracellular regeneration and occurs after the termination of infrasound impact.

Conclusions. 1. Infrasound with a frequency of 8 Hz and an intensity of 120 dB has a damaging effect on the structure of the myocardium, which is associated primarily with damage to cardiomyocytes, as well as with damages related to microcirculation process. In this case, the size of the damage increases with increasing of duration of impact.

2. Having a damaging effect on the myocardium, infrasound in parallel causes the development of compensatory and supportive processes, which can mask the clinical symptoms and thus impede the correct and timely diagnosis.

3. The concealment of the action of infrasound on the myocardium requires the timely detection of this harmful factor in production and the control of it for the sake of preserving the health of those who are exposed to its constant effect.

BIBLIOGRAPHY

1. Alekseev S.V., Glynchikov V.V., Usenko V. R. - Hygiene of Labor, 1983, № 8, p. 34–38.

2. Gabovich R. D., Shutenko O. I., Krechkovsky E. A. and others - Ibid., 1979, No. 3, p. 9—15

3. Karpova N.I., Alekseev S.V., Kadyskina E.N., Glinchikov V.V. – Works of Leningrad Sanitary and Hygienic Medical Institute, 1975, v. 114, p 24-29.

4. Karpova N.I., Malyshev, E N Low-frequency acoustic oscillations in the production. M., 1981.

5. Kosenkov I.M.. Pashkov V. A. - Works of Voronezh Medical Institute, 1975, v. 95. p. 85—87

6. Sanova A. G, - In the book: Fight against the harmful effects of noise and vibration. Kiev, 1976. p. 111 – 115.

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REACTION OF LIVER CELLS TO THE IMPACT OF INFRASOUND

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Intensive development of modern industry led to the creation of the machines and the mechanism of high power, which are the source of acoustic oscillations of various spectral range. The researchers pay special attention to infrasound as an integral part of production noises.

Scientific studies of recent years showed that infrasound in certain parameters has a harmful effect over the human body [1, 3, 7]. At the same time, the effect of infrasound is studied not only on the whole organism, but also on separate organs and tissues, as well as cellular structures [2,5]. Among the experimental works there are ones that show the harmful impact of infrasound over liver cells [4, 6]. However, many of the details of this process remained not researched and are the subject of this study.

The infrasound installation described in our previous work [3] was used at the experiment. The experiments were performed on sexually matured white rats-males weighing up to 250 g, which were exposed to infrasound frequency at 2, 4, 8, 16 Hz and with an intensity of 90–140 dB during 40 days with a daily exposure of 3 hours. The material was taken on the 5th, 10th, 15th, 20th, 40th day. The animals were decapitated. The material was fixed in 20% formalin, the cuttings were colored with hematoxylin-eosin, by Van-Gieson method, methyl green with pyronin, by Brache method and halo-cyanin, by Einarson method for nucleic acids

For electronic - microscopic research the pieces of liver were fixed by 2.5% glugraldehyde with additional fixation by 1% osmium and poured into araldide. Ultrathin cuttings were made on the ultratome LKB-III, contrasted by citrate of lead and studied in the electronic microscope JEM-7A.

It was established that infrasound has a damaging impact over hepatocytes of the liver at the frequency of 8 Hz and the intensity of 120 dB. In the glandular parenchyma of the liver there are diffuse changes which have the nature of reactive processes and are found in separate hepatocytes or in the whole group of cells. In addition, changes from the side of the sinusoid cells of the liver were observed.

The reaction of hepatocytes to the impact of infrasound was mosaic by nature and was expressed in the fact that the damaged cells lost contact with each other and were rounded. The phenomena of dissociation increased along with the effect of the infrasound and were characterized by changes from the side of both the nucleus and the cytoplasm. First of all, there was a deformation of the nuclei with the redistribution of chromatin and its concentration in the form of dense layer under the nucleus membrane. In the cytoplasm, the RNA content increased, it became sharply basophilic. Hepatocyte changes were more pronounced at the increase of the infrasound intensity up to 140 dB.

Electronic microscopic studies showed that mitochondrial swelling in reactively altered hepatocytes initially took place, the density of the matrix sharply increased, and deformation of the cristae was observed. The endoplasmic reticulum canaliculi expanded, and vacuoles of irregular shape and of various sizes were formed in them.

At long time exposure to infrasound, myelin-like bodies and lipid granules appeared in a number of hepatocytes on the 25th and 40th day.

In the granular cytoplasmic reticulum, the number of ribosomes sharply decreased and lysis areas appeared, especially around the nuclei (Fig. 1). The amount of glycogen decreased sharply compared with the norm. Around the lysis areas there were relatively small mitochondria with the dense matrix.

Next to sharply damaged hepatocytes there were cells in which nuclei chromatin was unevenly distributed, and in the endoplasmic reticulum there was a moderately pronounced vacuolization and the number of ribosomes decreased. Ultimately, in such reactively altered hepatocytes, the chromatin predominantly accumulates around the nuclear envelope, having the view of large clumps of irregular form. Vacuolization increased in the cytoplasm, but the swollen mitochondria contained shortened and fragmented cristae. Such hepatocytes remain viable after the termination of the infrasound action as well, gradually acquiring the normal structure.

The subject of degenerative changes are only those hepatocytes in which nuclear deformation takes place, but in the cytoplasm there are lysis areas with the ultimate formation of large vacuoles and the presence of small mitochondria with a dense matrix and destroyed cristae (Fig. 2). Polyblasts accumulate around dystrophicaltered hepatocytes and infiltrates gradually form. Proliferative processes are accompanied with the appearance of a large number of Kupffer cells, which are divided by mitosis and are accumulated in areas of the damaged parenchyma. In some cases, hepatocyte mitosis can be observed, which undoubtedly indicates the presence of regeneration processes.

The study showed that infrasound has a damaging impact over liver cells at a frequency of 8 Hz and an intensity of 120 dB, causing changes of both the nucleus and the cytoplasm. The initial form of the reaction of hepatocytes to the infrasound is the deformation of the nucleus with the redistribution of chromatin and the concentration of its clumps under the nuclear envelope with the disintegration of the nucleoli and the increase of the pernuclear spaces size. As a rule, such changes in hepatocytes are observed during the first day after irradiation with infrasound and are observed in those cells that are the subject of dissociation. At the same time, changes in the cytoplasm also take place in such hepatocytes, where mitochondria swelling with cristae fragmentation is observed.

Along with infrasound action, the number of reactively modified hepatocytes increases as well, especially on the 10-15th day, with the appearance of degenerative forms among them.

The greatest damaging effect of infrasound is observed at a frequency of 8 and 16 Hz and an intensity of 140 dB. At the same time, the number of dissociated hepatocytes increased, they formed whole groups. The nuclei of such cells were sharply deformed, and in the cytoplasm there were lysis areas of the endoplasmic reticulum, with ultimate formation of large vacuoles. In the preserved areas of the granular cytoplasmic reticulum, the canaliculi were enlarged and formed vacuoles of various dimensions and sizes. At the same time, lipid granules containing osmiophil inclusions appeared in the cytoplasm, and the structure of mitochondria changed.

The mitochondria that were located closest to the lysis area and were reduced in size, with a dense matrix and mild cristae, were altered most of all. In those areas of the cytoplasm in which the canaliculi of the granule network were preserved, though expanded, the mitochondria were enlarged in size, the fragmentation of the cristae was observed. The changes described above indicate that infrasound damages not only intracellular membranes and mitochondria, but also the nuclear apparatus, that can lead to the death of cells, if these changes have pathological nature and are accompanied by lysis of cytoplasmic areas with ultimate formation of large vacuoles.

Such hepatocytes ultimately die, and polyblasts and profiling Kupffer cells are accumulated around them.

Less damaged hepatocytes, in which lysis of the cytoplasmic membranes is not detected, are gradually restored, though the extended canaliculi of the endoplasmic reticulum and the increased density of mitochondria with moderate vacuolization remain in them for a long time.

BIBLIOGRAPHY

1. Andreeva-Galanina, E. Ts., Malyshev, E. N., Pronin, A. P., and Skorodumov, G. Ye. // Hygiene and Sanitation, - 1970. – no, 11 - p. 65-69.

2. Alekseev S. V., Kadyskin A. V., Kadyskina E. N., and others // The Impact of Vibration over the Human Body and Problems of Vibration Protection. - M., 1974. — P. 338-342.

3. Alekseev, S. V., Glinchikov, V. V., Usenko, V. R. // Hygiene of Labor.— 1983.— no. 8.— p. 34—38.

4. Gerlovin E. Sh., Alekseev S. V., Kadyskina E. N., Reiskanen A. V. // Issues of early diagnosis of professional diseases caused by the impact of physical factors. —L., 1978.— P. 91— 98

5. Erokhin V.N., Glinchikov V.V. // Noise, vibration and the fight against them at production. —L., 1979. — p. 92-93.

6. Prokopyeva, E. D.// Ibid., P. 208-210.

7. Reutov O. V., Erofeev N. P. // Works of Leningrad Sanitary and Hygienic Medical Institute. — 1976. — Vol. 114. — p. 14-16

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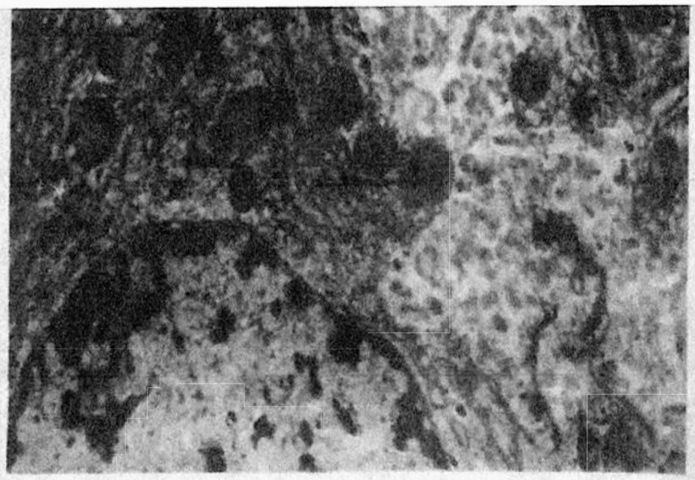

Рис. 1. Электронограмма гепатоцита через 5 сут после воздействии инфразвука.

Хроматин ядра имеет глыбчатую форму и в основном сконцентрирован около ядерной мембраны. В цитоплазме участ[ки] с разрушенной гранулярной сетью. Матрикс митохондрии уплотнен, кристы слабо выражены Ув. 12 000.

вакуолизация с постепенным уменьшением рибосом, появлением липидных гранул и уменьшением количества зерен гликогена, которые теряют четкость своих контуров и становятся менее электронно-плотными, чем в нормальных гепатоцитах. Наряду с изменением со стороны гепа[тоци]тов отмечаются изменения и в сосудах. Про[свет] последних переполняются кровью, а в не[кото]рых участках имеются и очаги кровоизли[яний]. Клетки синусоидов печени становятся базо[...]

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EARLY REACTION OF THE ORGANISM TO THE LOW-FREQUENCY ACOUSTIC OSCILLATIONS

Sanitary and Hygienic Medical Institute

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The causes of the appearance of the artificial infrasound are operating mechanisms with large radiating surfaces, as well as moving gas flows. According to a number of authors (V.I. Zinchenko and F.E. Grigoryan; E.N. Malyshev; Tempest; Hood and Leventhall, etc.) and the results of our researches, the sources of infrasound can be diesel engines, turbines, piston pumps, compressors, fans, large air blowing machines. Infrasound appears in tunnels for motor transport, in chimneys of high furnaces and in burners of open-hearth furnaces. The infrasound fields created by the work of the mentioned equipment have the intensity from 110 to 132 dB at the main frequencies 1,5, 2, 4, 8, 12 Hz.

Infrasound intensity often has a higher level than the level of audible sound. The distribution of infrasound, the degree of absorption by the atmosphere, the ability to disperse, etc. are somewhat different from the corresponding indicators of audible sounds. Infrasound can cause resonance of large objects due to the commensurability of the wavelength with these objects. All this causes some features of the impact of the infrasonic vibrations over the live organism and creates certain difficulties in combating them.

The Chair of Labor Hygiene of the Leningrad Sanitary and Hygienic Medical Institute performs the determination of the production sources of infrasound, clarifying the nature of its action over the organism, the determination of the changing mechanism observed in the organism under the influence of infrasonic vibrations. However, the disclosure of this mechanism is impossible without establishing the earliest reactions of the organism to the impact of the studying factor. This was the purpose of the present work.

The studies were conducted under the conditions of modeling the infrasound production parameters at the experimental acoustic complex specially equipped at the Chair. Recognized healthy men at the age from 19 to 29 years who passed preliminary medical examination took part at the research. In addition, the impact over the organism of infrasonic oscillations with the frequency of 5 and 10 Hz, with a sound pressure level of 100 and 135 dB was studied at rats, rabbits and guinea pigs. The time of factor action is 15 minutes Already in the first minutes of exposure, infrasound causes mental stress, vegetative reactions, unpleasant auditory sensations. The most common complaints caused by the infrasound action of the studied frequencies are feelings of general fatigue, lethargy and pressure in the ears. A small number of people (average 15–20%) had such symptoms as headache, dizziness, which were observed in a short time at the end and after the finalization of the experiment. More than half of the researched people complained for distraction, drowsiness, and feeling of depression. During the entire period of infrasound impact, some of the researched people noted the vibration of the internal organs, that mainly causes the sensation of vibrations at the chest, abdominal wall and stomach. These data allow to expect functional changes in the central nervous, cardiovascular systems, from the side of the hearing analyzer, respiratory system and vestibular apparatus under the influence of infrasound.

The functional state of the central nervous system was studied by electroencephalography method.

After a 15-minutes impact of infrasound, an increase in synchronization phenomena, most often in the left hemisphere, was observed at the electroencephalographic curves. In some cases, the hypsynchronized α-rhythm and the appearance of Θ-waves were observed in the left fronto-temporal region.

The obtained results allow us to make an assumption about the general reconstructions of the biopotentials, apparently caused by the impact of infrasound over the brain stem formations. These changes should be attributed to non-specific reactions associated with the weakening of the activating influences of the reticular formation of the trunk over the cerebral cortex (P. K. Anokhin; Moruzzi and Magoun, and others).

After the infrasound action with the frequency of 10 Hz, the intensity of 135 dB, the lengthening of the absolute values of the visual-motor reaction to the strong and weak stimuli and the decrease of the strength of the effector response were also observed.

At the action of infrasound with the frequency of 5 and 10 Hz and the intensity level of 135 dB, peculiar changes in the heart rhythm were noted. In the first minutes of exposure, the number of heartbeats tends to increase, expressed at the same level for both influencing frequencies. In 5–10 minutes, the heart rhythm slows down, returning to the initial, but after turning off the generator, the number of heartbeats becomes even more rare compared to the background values. Some studied people had an arrhythmia. These phenomena are most pronounced in the first minutes of the action of low frequencies, gradually disappearing with increasing of the time spent in the camera by studied people. A decrease in peripheral vascular tone was found, manifesting in the increase of skin temperature and in the decrease of maximum arterial pressure.

The study of cerebral hemodynamics was performed by rheoencephalography method. Analysis of rheoencephalogram showed that the action of infrasound is accompanied with the signs of inhibition of cerebral hemodynamics, manifested in the difficulty of venous outflow from the cranial cavity. The infrasound with frequency of 10 Hz, the intensity of 135 dB, caused deeper and more stable changes in the cerebral blood circulation, which consisted in a greater increase of the amplitude of the rheographic wave, in an increase of the duration of its anacrotic phase and in a decrease of the tonic voltage indicator compared to the impact of infrasonic vibrations with frequency 5 Hz of the same intensity. Under the influence of infrasound, the most noticeable and authentic changes in cerebral hemodynamics appear from about 7-10th minute of being in the infrasound field.

For registration of mechanical movements of the heart during contraction, the method of seismic cardiography developed by V. M. Baevsky and M. A. Kazaryan was used. The obtained results allowed to conclude that the infrasonic oscillations with intensity of 135dB cause disturbances in the mechanical movements of the heart, reducing the force of contraction of the heart muscle. This is manifested in a decrease in both the amplitude of the 1st oscillatory cycle, reflecting the magnitude of the cardiac forces acting during the systole, and the amplitude of the 2nd (diastolic) oscillatory cycle. The most pronounced changes in the contractile activity of the heart take place under the influence of infrasound frequency of 10 Hz.

Analysis of pneumograms registered during the action of infrasonic oscillations with a frequency of 5 and 10 Hz, an intensity of 135 dB shows changes in the respiratory function, manifested in the stable decrease of respiration frequency, starting from the 1st minute of the infrasound impact.

The state of the auditory analyzer was investigated with the help of tone audiometer AP-02. Researches of the infrasound impact with the frequency of 10 Hz and the intensity of 135 dB showed in most cases a slight exacerbation of hearing sensitivity - within 10 dB at the frequencies of 125, 250, 500 and 300 Hz.

The applying of the electron-syntagmography method did not reveal any disturbances in the vestibular apparatus under the influence of low-frequency oscillations of the studied intensity.

At experimental studies over the laboratory animals exposed to infrasound of the same parameters, changes in the bioelectrical activity of some cortical and subcortical structures of the brain, disturbances of redox processes in skeletal muscles, changes in the volume of nuclei of receptor cells in the helical body of the guinea pig snail were revealed that is a morphological expression of excitation caused by the infrasound action. Changes in the content of nucleic acids were found in these cells.

The results of the conducted researches allow us to conclude that infrasonic oscillations are not indifferent for biological objects, have the adverse effect over the entire organism and make many important functional systems react. The central nervous, cardiovascular, and respiratory systems, as well as the auditory analyzer are the most interested, reacting already in the first minutes of the infrasound impact. Among all studied parameters of infrasound, the deeper changes in the indicated systems of the studied people were caused by the oscillations with the frequency of 10 Hz and the intensity of 135 dB. The infrasound with the frequency of 5 Hz at the same intensity caused much smaller effect. Studies conducted at a lower infrasound intensity of 100 dB practically did not lead to the changes in the studied systems.

The analysis of the received data witnesses about the fact that the impact of infrasonic oscillations is manifested, primarily, in the violation of the mechanisms of central regulation of the body vital systems, the manifestation of which are the detected changes in the functional state of the cardiovascular and respiratory systems, violation of proteins synthesis and metabolic processes in the organism.

Thus, the study of the early reactions of the organism to the impact of infrasonic oscillations allows to reveal certain aspects of the mechanism of its biological action and contributes to the scientific argumentation of the production infrasound levels acceptable to the humans.

BIBLIOGRAPHY

Andreeva-Galanina, E. Ts., Malyshev and others . - Hygiene and Sanitation, 1970, no. 11, p. 65-69.

Anokhin P. K- Internal inhibition as a problem of physiology. M., 1958.

Baevsky P.M. Physiological measurements in space and the problem of their automation, M., 1970.

Zinchenko V.I., Grigoryan F.E. The noise of ship gas turbine installations. L., 1969.
Karpov N. I. and others. - Hygiene of Labor, 1972, No. 7, p. 36-38.

Eigkman E., Vendrik A.J. - J. Acoust. Soc. Amer., 1960, v. 32, p. 1134-1139.

Evans M.J., Tempest W. - J. sound. Vibration, 1972, v. 22, p. 19-24.

Haad R. A., Lewenthall H. Z. - Acoustics, 1971, v. 25, p. 10113.