

# Negative Effect of High-Level Infrasonnd on Human Myocardial Contractility: In-Vitro Controlled Experiment

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## Abstract

**Background:** Human exposure to infrasound is increasing due to man-made factors, such as occupational conditions, wind farms and transportation. The concern among the public regarding the safety of infrasound exposure is growing. **Aims:** To evaluate whether exposure to infrasound interferes directly with human cardiac function and contributes to pathological processes. **Setting:** The University Hospital of Mainz, Germany. **Methods:** Human myocardial tissues, obtained from patients undergoing cardiac surgery, were prepared in small muscle samples and stimulated electrically in-vitro for a period of almost two hours under physiological conditions to induce continuous pulsatile contractions and simulating a working human heart. Two samples were obtained from each donor: one was subjected to infrasound for 60 min and the other served as a control. Their contraction forces (CF) and durations (CD) were measured before and after each testing period and their relative changes (CF% and CD%) were calculated and introduced in a multilinear regression model. The following three infrasound levels of exposure were used in this study: 100, 110 and 120 dBz. **Results:** The measured CF% corresponded negatively with the infrasound level measured in dBz ( $R^2 = 0.631$ ;  $P = 0.018$ ). The decrease measured almost  $-11\%$  at 110 dBz and  $-18\%$  at 120 dBz, after correction for control. The CD on the other hand remained unchanged. **Conclusions:** Exposure to high levels of infrasound (more than 100 dBz) interferes with cardiac muscle contractile ability, as early as one hour after exposure. There are numerous additional studies which support this conclusion. These results should be taken into account when considering environmental regulations.

**Keywords:** Environmental legislation, heart, infrasound, laboratory researchkey messages

## Key Messages

Environmental regulations should be reconsidered to set a maximum tolerated level of chronic exposure to infrasound no higher than 90 dBz, as higher level can interfere with the cardiac function

## INTRODUCTION

Infrasound is a common phenomenon existing widely in nature and produced in numerous ways, such as wind and thunder. Modern society has greatly increased its generation through man-made sources, such as occupational conditions, industrial installations, vibration of mechanical equipment inside enclosed spaces (like heating and ventilation systems), wind turbines and transportation.<sup>[1-2]</sup> Opening the rear window in a car traveling at 100 km/h for example, exposes the passengers to levels of infrasound as high as 125 dBz.<sup>[1]</sup> This increase in human exposure to infrasound is

historically unanticipated and has led to growing concern among the public regarding its safety.<sup>[3]</sup> This concern has been compounded by a wide spectrum of complaints, which have been reported worldwide among populations exposed to infrasound. Symptoms attributed to the effect of infrasound include, but are not limited to, headache, concentration deficit, mood change, depression, sleep disorders, pulsation and panic disorders, especially between individuals, who are exposed chronically, due to occupational conditions or by

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1 their domicile residing near industrial sources.<sup>[4-5]</sup> People  
 2 experiencing effects from infrasound often describe it as a  
 3 deep humming vibration in their body, or in external objects  
 4 around them.<sup>[4]</sup> Some individuals even report being able to  
 5 hear it, which sheds light on the common misconception  
 6 regarding the audibility of infrasound, as many individuals,  
 7 including professionals, equate infrasound (acoustical  
 8 frequencies less than 20 Hz) to be inaudible sound. In fact,  
 9 it has been shown as early as the 1930s that infrasound can  
 10 still be perceived when the pressure level is high enough.<sup>[4,6-8]</sup>

11 Several experimental and environmental studies have  
 12 suggested the association between infrasound and negative  
 13 effects on public health.<sup>[3,9-11]</sup> Many animal tests found  
 14 infrasound to negatively affect the heart,<sup>[12]</sup> liver,<sup>[13]</sup>  
 15 nervous system<sup>[14]</sup> and the lungs.<sup>[15]</sup> However, it is still  
 16 not known to what extent such negative effects happen in  
 17 an everyday-life environment. Also, the exact mechanism by  
 18 which infrasound affects human health, including which  
 19 organs are especially at risk, is still common topic of  
 20 discussion.

21 Cardiac function is the result of a very finely tuned  
 22 mechanical system, requiring continuous cyclic interaction  
 23 between actin and myosin to produce a powerful contraction,  
 24 enabling the pumping of blood throughout the body. It is still  
 25 unclear what effects the high energy levels of infrasound  
 26 (acoustic vibration below 20 Hz) have on the heart.

27 This research attempts to answer this question by  
 28 investigating the effect of exposure to high levels of  
 29 infrasound on cardiac contractility. The first objective in  
 30 this study is to evaluate whether or not infrasound affects  
 31 heart muscle tissue. The second objective is to attempt to  
 32 quantify this effect, if it exists, and to extrapolate its relevance  
 33 in the modern-day environment. To accomplish this, we  
 34 subjected human cardiac tissue obtained from the right  
 35 atrium to infrasound directly in an in-vitro model, isolating  
 36 the tissue from all other factors interfering with its function.  
 37 We used three different energy levels of exposure (100, 110  
 38 and 120 dBz). These energy levels fall relatively in a gray  
 39 zone, since levels above 120 dBz are well known to be  
 40 dangerous to humans.<sup>[11]</sup>

## 41 METHODS

### 42 Ethics approval

43 This study was conducted after obtaining clearance from the  
 44 Ethics Board of Rhineland-Palatinate, Germany. We obtained  
 45 individual written consent from patients for the use of  
 46 disposed tissue arising from the surgical procedure(s), with  
 47 the assurance of anonymity. No personal information was  
 48 collected in this study.

### 49 Experimental tissue and preparation

50 Tips of the right atrial appendages that were routinely  
 51 removed and discarded from patients undergoing cardiac  
 52 surgery during the establishment of a cardiopulmonary

bypass were collected. Tissues were excluded in the  
 presence of the following condition: age >90 or <18  
 years; severe cardiomyopathy, defined as an ejection  
 fraction (EF)  $\leq$  30%; inflammatory or infective cardiac  
 disease (e.g., endocarditis); congenital malformations;  
 surgery for pathologies involving the right atrium, for  
 example, tricuspid regurgitation; digitalis therapy; and the  
 history of atrial fibrillation or flutter. Standard cardiovascular  
 anesthesia was applied using total intravenous protocols with  
 propofol and remifentanyl. Noradrenaline, physiological  
 solutions for volume substitution and atropine were  
 frequently used as required.

13 The samples from 18 patients were transported immediately  
 14 after the surgical excision to our laboratory in a cold (4°C)  
 15 modified Bretschneider cardioplegic solution (MBSC,  
 16 prepared by the pharmacy of the University Medical  
 17 Center of the Johannes Gutenberg University, Mainz,  
 18 Germany), which contained 15 mM NaCl, 10 mM KCl,  
 19 4 mM MgCl(H<sub>2</sub>O)<sub>6</sub>, 18 mM Histidine.HCl.H<sub>2</sub>O, 180 mM  
 20 Histidine, 2 mM Tryptophan, 30 mM Mannitol and  
 21 0.015 mM CaCl<sub>2</sub>(H<sub>2</sub>O)<sub>2</sub> and has a pH-value of 7.2  
 22 (25°C). They were manually prepared under the  
 23 microscope to yield muscle specimens measuring  
 24 3 × 0.5 × 0.5 mm<sup>3</sup> [Figure 1]. Following, these specimens  
 25 were stored in dark cold (4°C) oxygenated MBS solution  
 26 for 1–24h, before being used in experiments.

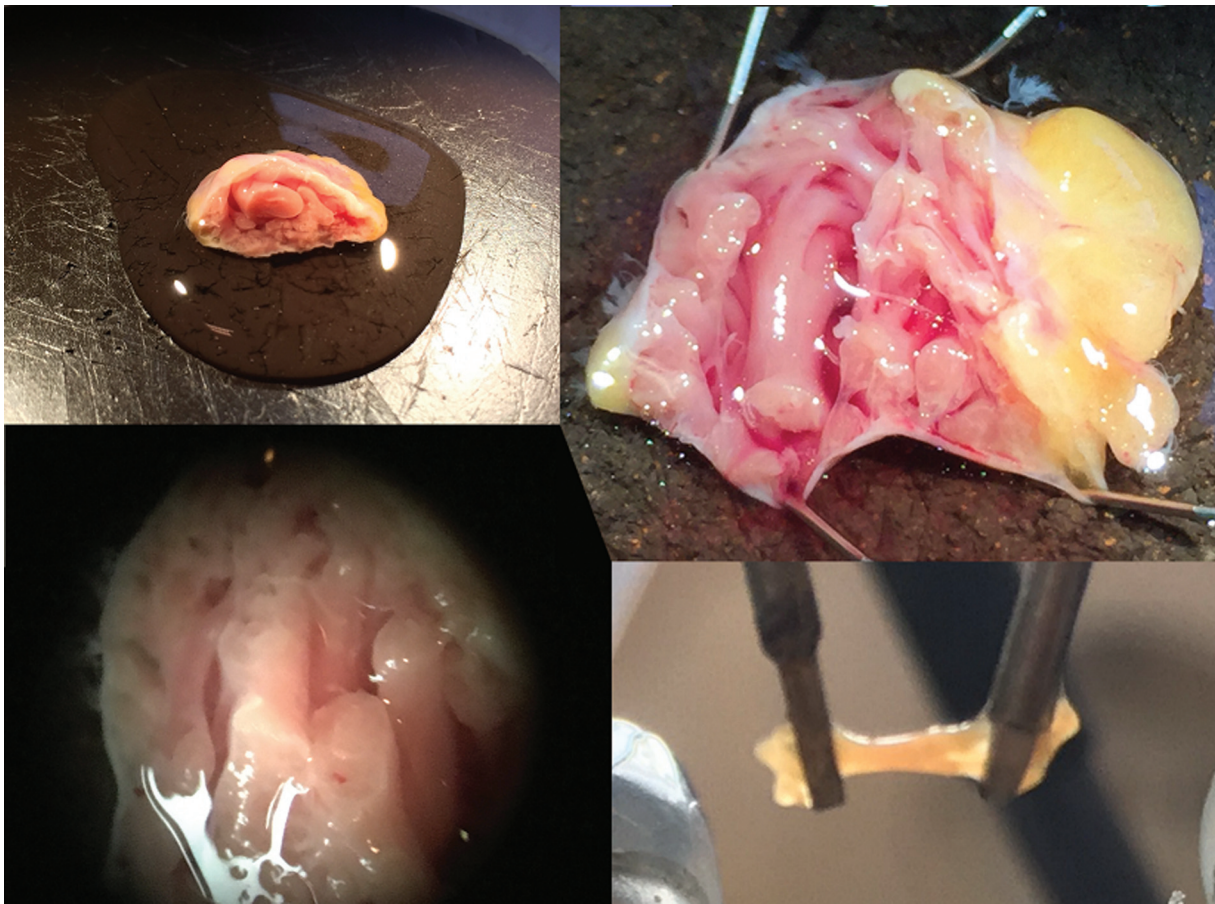
### 53 Infrasound application

54 A 30 cm Woofer was connected to a power amplifier, to a  
 55 computer, and fixed at the top of a special made closed  
 56 chamber, where the muscle investigation system was  
 inserted. A feedback loop, consisting of measurement  
 microphone (calibrated Superlux ECM999) and  
 microphone amplifier was connected back to the computer.  
 A special software (TrueRTA Audio Spectrum Analyzer),  
 was used to generate a pure sinusoidal 16Hz signal and to  
 analyze the measurement, verifying the exposure level.  
 Figure 2 illustrates this design.

Three sets of trials were conducted using three different  
 infrasound levels: 100, 110 and 120 dBz. Infrasound  
 measurement was conducted using no weighting (known  
 as Z-weighting or linear weighting).

### 57 Trial preparation

58 At the beginning of each experiment, every specimen was  
 59 washed with the Krebs–Henseleit (KH) buffer – which  
 60 contained: 118 mM NaCl, 25 mM NaHCO<sub>3</sub>, 4.6 mM KCl,  
 61 1.2 mM KH<sub>2</sub>PO<sub>4</sub>, 1.2 mM MgSO<sub>4</sub>, 1.3 mM CaCl<sub>2</sub> and  
 62 11 mM glucose – and warmed for approximately 10 min. It  
 63 was then mounted horizontally between two tweezers of a  
 64 muscle investigation system (modified ‘Standard System for  
 65 Muscle Investigation,’ SH Heidelberg, Heidelberg,  
 66 Germany) and exposed to a continuous flow of warm  
 (35°C) KH buffer, steamed with a mix of 95% oxygen and  
 5% carbon dioxide at a rate of 0.5 mL/min. After a precise



**Figure 1:** Preparing the atrial muscle specimens. The right atrium appendage (top left) is fixed with needles (above right) to prepare the Trabeculae carneae manually and carefully under the microscope (bottom left) to get strips measuring  $3 \times 0.5 \times 0.5 \text{ mm}^3$ , which are fixed between two tweezers of the muscle investigation system (bottom right).

baseline length measurement, it was stretched to 110% of its slack length. Next, electrical stimulation (field stimulation) was applied at a frequency of 75 bpm. The voltage was gradually increased from 1 V to a maximum of 10 V, until the maximal contraction force (CF) of the specimen was reached. Following, it was left to stabilize for 30 min to reach a steady state before starting the experiment.

### Experiment design

Two samples, obtained from the same patient, were stimulated simultaneously in each experiment with only one of them exposed to infrasound and the other serving as a control. Two identical muscle investigation systems were used for this purpose. Each experiment was conducted using samples from a different donor. The exposure to infrasound lasted 60 min. The samples were allowed to beat for another 30 min before ending the experiments. Figure 3 explains the design used for this study.

### Data analysis and statistical assessment

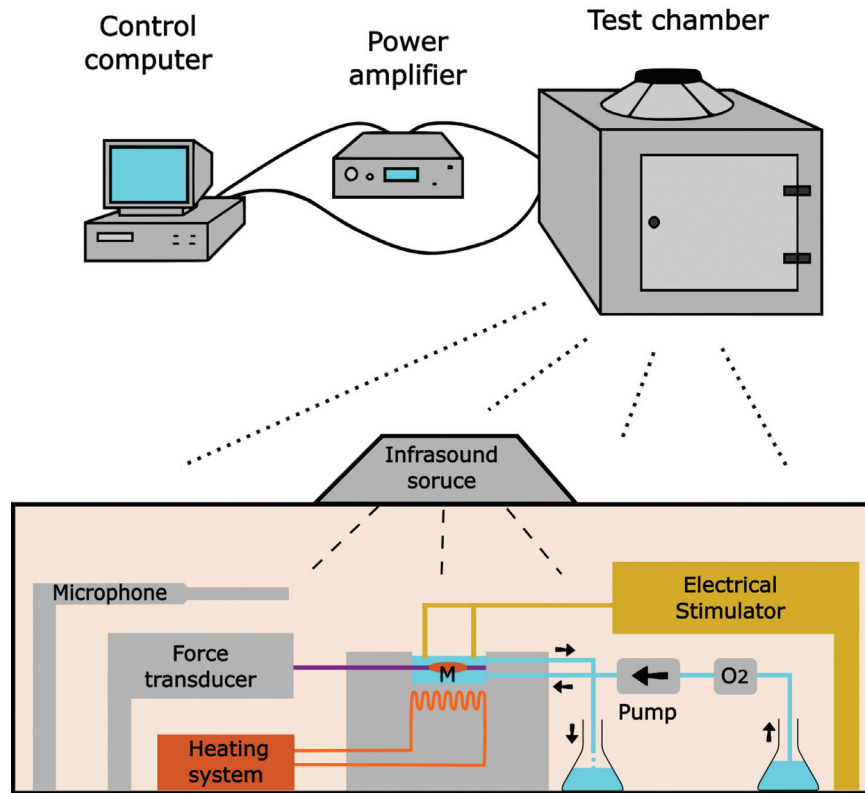
We relied on the “PicoScope 2204A Pico Technology, Cambridgeshire, UK”, using the “PicoLog Software” for data acquisition and recorded the complete trials as plain

“txt” files. The data were then processed with Excel 2016 (Microsoft Corp., Redmond, WA, USA). A self-developed Macro (available as supplement) was used to calculate the two variables measured in this study: the contraction forces (CF) and duration (CD). Figure 4 provides an optical explanation for the calculation process. The measurement was repeated twice: directly before applying the Infrasound ( $CF_1$  and  $CD_1$ ) and after ( $CF_2$  and  $CD_2$ ). Each measurement lasted 10 min and the average values were recorded. Following, the relative changes ( $CF\%$  and  $CD\%$ ) were calculated according the following equation:

$$CF\% = 100 \times CF_2 / CF_1$$

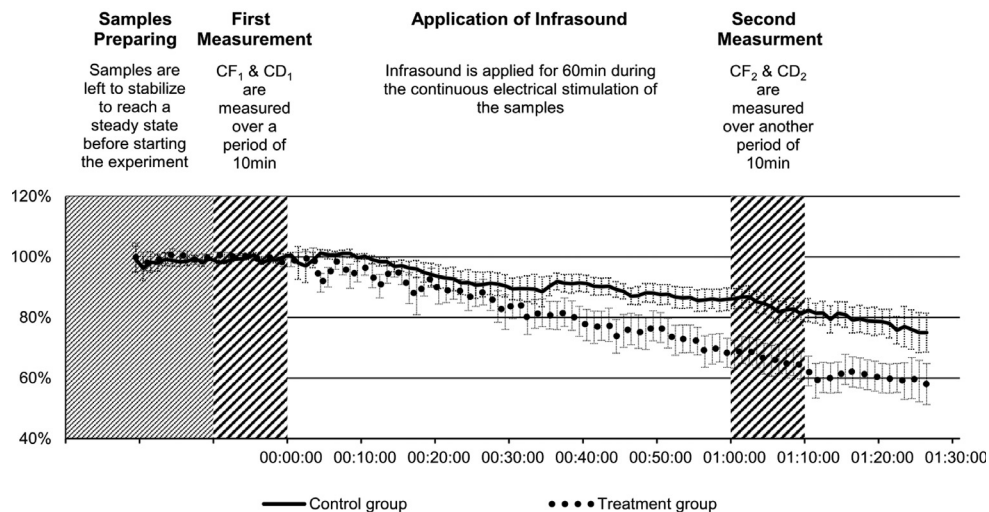
Using the relative changes in the statistical evaluation, instead of the absolute values was necessary to adjust for the diversity of the muscle specimens and their initial forces, which resulted from the manual preparation. This approach was found justifiable as this research was only interested in studying the changes induced by the infrasound and not the samples themselves. As those ratios tend usually to contradict the gaussian distribution and follow an exponential pattern, we performed a logarithmic transformation, before finally analyzing their logarithms using a *multiple linear*

## Experimental setup



**Figure 2:** A computer is connected to a power amplifier to an infrasound woofer, which is mounted at the top of the test chamber, which is specially constructed to allow levels of infrasound up to 130 dBz and houses the muscle investigation system inside of it. A measurement microphone is included to ensure a steady level of infrasound throughout the experiments. The cardiac sample (M) is brought inside, where it is electrically stimulated to perform pulsatile contraction at a frequency of 75 bpm, simulating a working human heart. The contraction force and duration are measured then.

### Illustration of the experiment design with a continuous recording of contraction force (CF)



**Figure 3:** Experiment design: First the samples are stimulated for a period of 30min until they reach a steady state. Then the CF and CD are measured over a period of 10 min (CF<sub>1</sub> and CD<sub>1</sub>). Infrasound is applied for a period of 60 min during the continuous electrical stimulation to only one of the two samples. The second one serves as a control. At the end, the measurement is repeated over a further period of 10 min (CF<sub>2</sub> and CD<sub>2</sub>). Lastly, the relative changes (CF% and CD%, the ratio between the values after the treatment and before) are calculated for both the test and control samples.

Recording and calculation of CF & CD

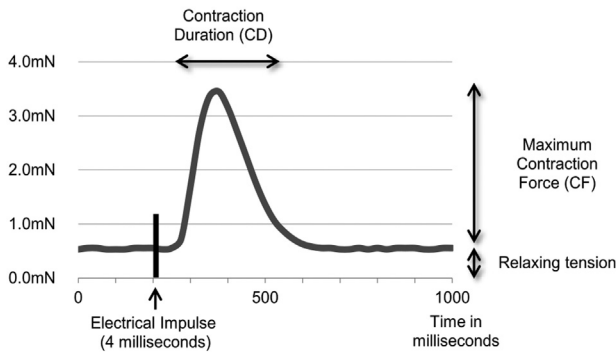


Figure 4: The maximal isometric contraction force (CF) and duration (CD) were calculated directly from the continuous registration of force.

Table 1: Summary of the medical profiles and medications of the patients (n = 18)

Medical situations, treatments and medications	Frequency	Ratio (%)
Aortic valve replacement	8	44
Mitral valve repair/replacement	4	22
Coronary artery bypass graft	11	61
Coronary artery disease	12	67
Atrial fibrillation	3	17
Severely reduced ejection fraction	0	0
Moderately and slightly reduced EF	2	11
Diabetes mellitus	9	50
Mitral valve insufficiency	6	33
Arterial hypertension	17	94
Renal insufficiency	1	6
Aspirin	9	50
Beta blockers	11	61
Statins	9	50
Diuretics	7	39
Metformin	3	17
Thyroxine replacement therapy	2	11
Antihypertensive medications	14	78

regression model. We considered  $CF\%_{test}$  (the relative change in the corresponding test sample) as a dependent variable and both the corresponding  $CF\%_{cont}$  (the relative change in the corresponding control sample) and  $infrasound\_level$  as the explanatories. Values were finally reported as ratios for clarity.

Statistical Analysis was done using XLSTAT Statistics. Descriptive variables were described by frequencies and quantitative variables by mean. We verified the normality of the measured values using the *Anderson-Darling Test*. The sample size needed in this work was determined after conducting primary trials, using the same model. By setting the statistical power to 0.8, six trials were needed for each group. This decision was also augmented by our experience with this model in previous researches.<sup>[16]</sup> Only one-tailed *P*-value was computed as we expected a negative effect of Infrasound based on our literature research. An *a*-value of 0.05 was chosen for significance.

## RESULTS

Six trials for each of the three groups were conducted. The average of age of the 18 donors was  $67.8 \pm 8$  years (mean  $\pm$  SD), eight of which were female and ten were male. Table 1 illustrates their patient profile. No significant differences in patient characters were seen between the three groups.

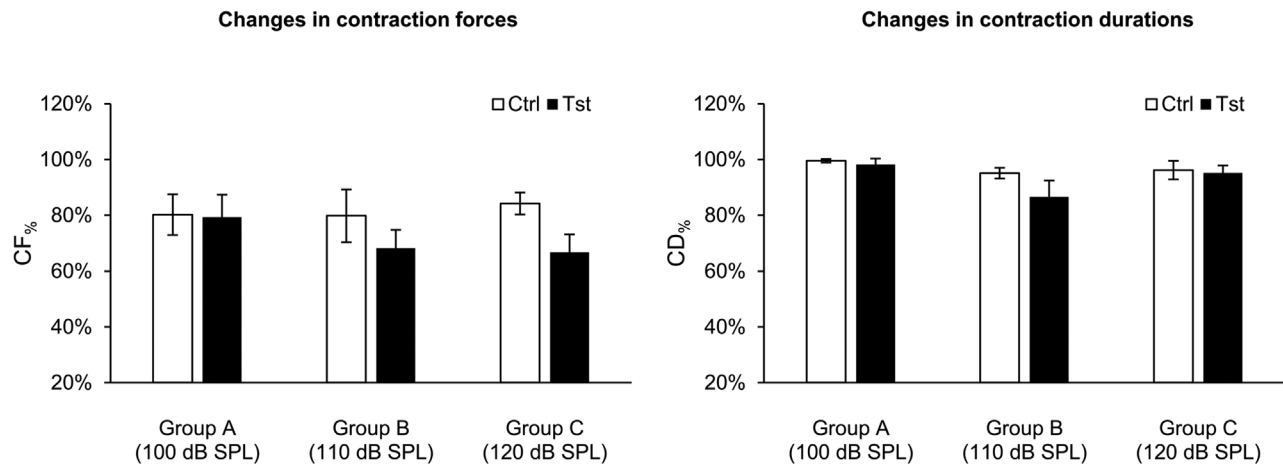
The measured  $CF\%_{test}$  (treated with infrasound) were found to correlate positively with the  $CF\%_{cont}$  ( $p = 0.0003$ ) and negatively with the  $Infrasound\_level$  ( $p = 0.018$ ). The  $CF\%_{test}$  measured almost  $-11\%$  less than the  $CF\%_{cont}$  at 110dBz and  $-18\%$  at 120dBz (after correction for  $CF\%_{cont}$ ). The following predicting model was calculated ( $R^2 = 0.631$ ;  $P = 0.0006$ ):

$$\text{Log}(CF\%_{test}) = 0.892 - 0.0048 \times \text{Infrasound\_level} + 0.778 \times \text{Log}(CF\%_{cont})$$

Exposure to infrasound did not alter the contraction duration (CD) in any group ( $P = 0.765$ ). Table 2 and Figure 5 illustrate the results.

Table 2: The measured CFs of all trials in millinewton with their relative changes (CF%)

Trial	Contraction Force (CF) in millinewton																	
	Group A: 100 dBz				Group B: 110 dBz				Group C: 120 dBz									
	Control		Test		Control		Test		Control		Test							
Nr.	Bef	Aft	Bef	Aft	Bef	Aft	Bef	Aft	Bef	Aft	Bef	Aft						
1	1.7	1.6	92%	2.4	2.4	99%	2.8	2.4	84%	1.4	1.2	82%	1.6	1.3	80%	0.8	0.5	65%
2	2.0	1.6	79%	1.3	0.9	64%	1.4	1.2	85%	2.2	1.3	61%	2.0	1.7	88%	2.8	2.4	86%
3	1.6	1.2	75%	1.4	1.1	78%	1.4	1.3	94%	3.6	3.1	85%	1.6	1.3	80%	1.0	0.9	82%
4	1.5	1.4	94%	2.0	1.7	86%	0.8	0.3	40%	0.3	0.1	41%	2.1	1.8	87%	1.3	0.8	64%
5	2.9	2.7	94%	3.3	3.2	99%	2.3	2.5	107%	1.3	1.0	72%	1.0	1.0	99%	0.6	0.3	61%
6	0.9	0.4	47%	0.4	0.2	49%	2.1	1.5	69%	2.2	1.5	69%	2.8	2.0	71%	1.7	0.8	43%



**Figure 5:** The measured contraction forces (CF) after exposure to infrasound were negatively corresponded to Infrasound\_level measured in dBz ( $P=0.018$ ). The  $CF_{\%\_test}$  decreased almost 11% against the  $CF_{\%\_cont}$  at 110 dBz and 18% at 120 dBz. Exposure to infrasound did not alter the contraction duration (CD) in any group ( $P=0.765$ ).

## DISCUSSION

This study shows a strong negative effect of exposure to high level of infrasound (above 100dBz) on the contractility of cardiac tissues in-vitro. This finding is unique, as it is the first evidence to demonstrate such a direct effect of infrasound on the cardiac function in humans. The measured effect of almost 9% decrease in contraction force for every 10 dBz above 100 dBz is relevant, especially when considering that this effect was observed after only one hour of exposure.

Interpreting the significance of this finding in an everyday environment requires some clarification regarding the physical character of infrasound and its effects on the whole body protecting the human heart. Infrasound is the extension of the audio spectrum, when the frequency falls below 20 Hz. As a result, it shares much with the audible spectrum, but with some unique characteristics. The very long wavelength (considering the acoustic velocity of 343m/s at 20° dry air sea-level, there is a wavelength of more than 17.5 m) compared to the audible sound, enables infrasound, by means of reflection; refraction and diffraction, to pass through and around different obstacles, such as buildings and terrains. The long wavelength also allows infrasound to maintain energy, remaining relatively stable after traveling long distances. For the same reason, common noise barriers are usually ineffective against it. It is also the same reason why it is usually not a simple procedure to locate infrasound sources, even when many individuals, who describe a feeling of a drum in their entire body, easily perceive it.

It is also common for infrasound to generate high energetic standing waves in enclosed spaces, when the space dimensions are multiples of the half wavelength of some externally or internally presented infrasound signal, increasing the infrasound level further by condensing its energy by means of resonance.<sup>[17]</sup> This kind of resonance, also known as *Helmholtz resonance*, sometimes leads to infrasound increasing inside of residential rooms with open

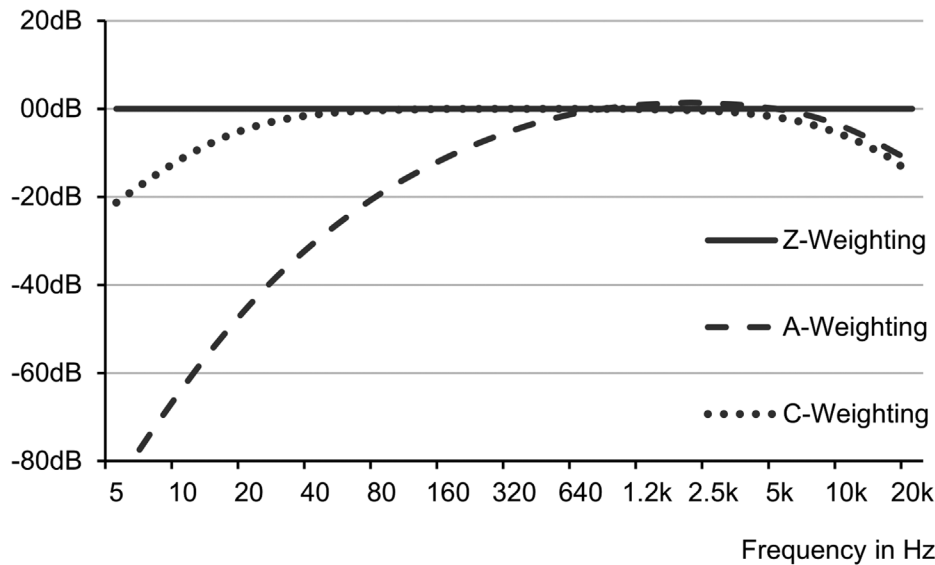
windows, or through ventilation ducts and affecting people by reaching levels up to 25dBz higher than the measured level outside.<sup>[18]</sup> It also partially explains why some people may complain about infrasound without even being in the direct vicinity of its sources, with other individuals not perceiving effects at all, and why the complaints are often about indoor disturbance instead of outdoor.<sup>[4]</sup> For example, while some outdoor measurements may read a level of 80 dB, at the same time in a nearby living room 100 db can be present.

The human body itself does not shield against infrasound. In contrast, it may emphasize it by mean of resonance, as it has been shown that the upper human torso tends to resonate between 5 and 250 Hz.<sup>[19-20]</sup>

An area needing clarification is the ambiguity inherent in the measuring methods presently used regarding audible noise and infrasound. While most legislation and regulations specify the maximum tolerated noise level using the A-weighting system, it is important to define the nature of this system. The A-weighted acoustic measuring method is specifically designed to diminish the inaudible part of the acoustic spectrum. As a result, an exposure to a high-level 100 dBz infrasound signal with a frequency of 16 Hz would measure merely 45dB<sub>(A)</sub>, deeming it acceptable according to many of the present-day noise regulations. Figure 6 clarifies this weighting system.

Epidemiological studies regarding infrasound are usually difficult to conduct and are often inefficient. People under infrasound exposure may not notice it as infrasound, since it is not usually audible or perceptible, which may lead them not to participate in such studies. The ability to sense or hear infrasound is extremely subjective, exaggerating its unpleasant presence by some individuals.<sup>[11]</sup> In contrast to epidemiological studies, laboratory research has been conducted extensively regarding infrasound, especially during the 1970s and 1980s in the Soviet Union with many interesting results, which need to be discussed here.

## Acoustic Weighting Systems



**Figure 6:** Acoustic measurement according to the DIN EN 61672-1:2014-07 standard. <sup>[21]</sup> Noticed how applying the A-weighting attenuates the signal in the low-frequency region up to more than 80 dB, as compared to the Z-weighting (no filter). C-weighting is another commonly used filter, which falls in between.

The workgroup of Karpova *et al.*<sup>[22]</sup> concluded that levels of 110 to 132dB at frequencies of 1 to 12Hz are possible near sources such as diesel engines; turbines; piston pumps; compressors; fans and other large air blowing machines. They went further to test the effect of these levels on healthy young men at age from 19 to 29 years and found them to cause mental stress; vegetative reactions and unpleasant auditory sensation, already after the first minute of exposure. Further symptoms were a feeling of general fatigue, lethargy, pressure in the ears, dizziness, distraction, drowsiness and feeling of depression during the next 15 min of exposure. By examining the effect on the heart, the same group noticed changes in cardiac rhythm and arrhythmia. Using an old technique known as the seismic cardiograph, they also noticed a reduction in the force of contraction of the heart muscle, with the most pronounced changes induced by frequency of 10 Hz. Another soviet workgroup, Gordeladze *et al.*<sup>[12]</sup>, examined the effect of infrasound at a frequency of 8 Hz and intensity of 120dB on white rats and guinea pigs.<sup>[12]</sup> As soon as after 3 hours of exposure, they noticed pallor and swelling of the left and right ventricular walls with small-point hemorrhages in the pericardium. Microscopically, mitochondrial swelling, with destruction of outer membranes and endothelial swelling were noticed. After a day of exposure, the activity of redox enzymes had fallen; the sarcoplasm of cardiomyocytes was edematous; the sarcolemma was damaged in a number of areas and the mitochondrial swelling continued to exist. After 5 days cardiomyocytes began to show signs of granular dystrophy; the activity of redox enzymes was reduced; the

myofibrils were fragmented in the areas of the discs; the mitochondria were swollen with cristae being finely fragmented; the erythrocytes accumulated in the lumens of the dilated capillaries and the swollen endothelial cells included destroyed mitochondria. Changes in the nuclei were noticed after 25 days with rugged contours; chromatin located in the form of clumps of various sizes and enlarged pores. Full restoration of damaged cardiac cells was noticed after the termination of infrasound exposure though. Similar results were noticed by the same team in another work studying the effect of infrasound on the liver cells.<sup>[23]</sup> Using the same experimental setup, they noticed resembling changes in both the cytoplasm und the nucleus, with redistribution of chromatin and concentration in the form of dense layer under the nucleus membrane and increased RNA content. Myelin like bodies and lipid granules appeared in a number of hepatocytes on the 25th and 40th day. There was also a decrease in the number of ribosomes. The Mitochondria were swollen and contained shortened and fragmented cristae. Obviously, the infrasound damaged not only intracellular structure and mitochondria, but also the nuclear apparatus.

There is plenty of evidence regarding the damaging effect of infrasound upon the heart. After exposing Sprague-Dawley rats to 5Hz infrasound at 130 dBz for 3 hours daily, Pei *et al.*<sup>[9]</sup> found changes in cardiac ultrastructure, hemodynamics indices, intracellular  $Ca_2^+$  concentrations and sarcoplasmic reticulum  $Ca_2^+$ . Further, the heart rates increased significantly in comparison to a control group in

the first day of exposure. Maximum dropping rates of left ventricular pressure (corresponding to the diastolic function) were significantly decreased. There were also several swollen mitochondria and platelet aggregation in the intercellular space of the exposure group, the same finding which has been previously reported by Gordeladze *et al.*<sup>[12]</sup> and Alekseev *et al.*<sup>[24]</sup> in the 1980s. Prolonged exposure altered the L-type  $Ca_2^+$  currents as well.<sup>[25]</sup> Pei *et al.*<sup>[26]</sup> also investigated the apoptotic effect of infrasound on neonatal rat cardiomyocytes by exposing them to 5Hz at 130dB for several days and found that infrasound induces apoptosis in a time-dependent manner. The expression of proapoptotic proteins such as Bax, caspase-3, caspase-8, caspase-9 and FAS was significantly up-regulated, with concomitant down-regulated expression of antiapoptotic proteins. Another underlying mechanism for the damage induced by infrasound is the oxidative stress, which was also investigated by the same team, who found the expression of CAT, GPx, SOD1 and SOD2 and their activities in rat cardiomyocytes in infrasound exposure groups were significantly decreased compared to controls, along with significantly higher levels of  $O_2$  and  $H_2O_2$ .<sup>[27]</sup> Further, Lousinha *et al.*<sup>[28]</sup> showed that exposure to 90 to 145dB infrasound induces coronary perivascular fibrosis in rats. It is worth mentioning here that Pei *et al.* used very high level of Infrasound (130dB) in their experiments to induce these effects. Besides, they exposed cardiomyocytes in their experiment directly to infrasound, without a protection of surrounding tissues. Whether cardiomyocytes are ever exposed to such levels of infrasound in in-vivo and in real world environment remains questionable. On the other hand, examining the effect of moderate, chronic, real-world exposure to infrasound over an extended time period is much more challenging and is not feasible in laboratory conditions.

Infrasound also can exert a negative effect on the cardiovascular system in an indirect way. As we previously discussed, many people can hear it or perceive it through their body and for them it is another form of noise, which is associated with mental stress. It is currently well known that noise can cause oxidative stress; vascular dysfunction and inflammation, resulting in adverse cardiovascular effects and ultimately leading to cardiac remodeling and fibrosis.<sup>[29-32]</sup>

Wind turbines are being built faster than ever, invading new geographic locations every day, further increasing potential exposure to infrasound. They are usually well accepted with positive attitude toward them, being a source of green energy and helping in reduction of atmospheric carbon dioxide with no further known gaseous emissions.<sup>[33]</sup> Nevertheless, people on a local level and residents in their immediate vicinity sometimes oppose them. These individuals are frequently reporting annoyance, headache, concentration difficulty, irritation and sleep disorders.<sup>[11,34]</sup> Similar complaints, like drowsiness, numbness, ear pressure, nausea and breath depression, are well described under

laboratory conditions and after a short exposition to high level of infrasound.<sup>[10,35-36]</sup> Thus, it seems reasonable to attribute some complaints about wind turbines to the infrasound radiated by them. Disagreement exists though regarding the exact level of infrasound emission by wind turbines and its geographical extent. The tendency toward building larger wind turbines to achieve more electrical power is ongoing, with a multitude of projects being currently under consideration or construction worldwide. Whether or not wind turbines are, or will be, able to produce harmfully high levels of infrasound, levels that are associated with pathological changes similar to those previously discussed, remains out of the scope of this paper. However, with all the physical effects discussed above and as medical researchers, it is strongly recommended to conduct adequate physical examinations and measurements under real world conditions to assure that infrasound levels generated by wind turbine farms do not approach pathological levels. The researchers **of for** this article recommend setting the level of generated infrasound as low as 80 dBz (20 dBz below the critical value of 100 dBz) as the maximally tolerated limit for chronic exposure; this recommendation is similar to the 85 dB<sub>(G)</sub> level recommended by the Danish Environmental Protection Agency in 1997.<sup>[37]</sup>

Finally, the following points need to be considered. The myocardial samples **used** were obtained from a typical cardiac-surgery population, consisting of elderly individuals with a variety of cardiac pathologies. There are known differences between atrial and ventricular myocardium, such as approximately 15% smaller atrial cell volume yielding higher surface-to-volume ratio; smaller amplitude of systolic  $Ca_2^+$  transients; accelerated rates of decline of systolic  $Ca_2^+$ ; more sarcoplasmic reticulum (SR) mediated  $Ca_2^+$  uptake and higher SR  $Ca_2^+$  content.<sup>[38]</sup> Additionally, a higher density of mitochondria is found usually in ventricles.<sup>[39]</sup> However, these differences are merely quantitative and do not constitute new mechanisms or pathways.<sup>[40-41]</sup> Hence, it is acceptable to use the atrial tissues in our investigation, especially when the target is screening for possible effects.

## CONCLUSION

Exposure to high level of infrasound (more than 100 dBz) negatively interferes with cardiac function, even as soon as one hour after exposure. Numerous independent laboratory research from around the globe has been performed, resulting in similar findings supporting this conclusion. The effect of infrasound goes obviously beyond the direct mechanical effect in increasing the cross-bridge breakage and involves a wide range of processes, such as calcium metabolism and mitochondrial integrity. These results should be considered when looking at environmental regulations. It is the recommendation of this research group to set the level of infrasound no higher than **90dBz** as the maximally tolerated limit for chronic exposure.



## DISCLAIMER

### Ethics approval

All procedures performed in this study involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. This study was performed with the permission of the Ethics Board of Rhineland-Palatinate, Germany.

This article does not contain any studies with animals performed by any of the authors.

### Informed consent

Informed consent was obtained from all individual participants included in the study.

### Consent to publish

Not applicable.

### Availability of data and material

The datasets used during this study are available as supplements and from the corresponding author upon reasonable request.

### Conflicts of interest

There are no conflicts of interest.

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The corresponding author confirms that all authors have read and approved this manuscript.

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Author Queries???

AQ1: Please provide author given name [Miljøstyrelsen].