



Research article

Effects of high-intensity infrasound on liver lipid content of rats

Gonçalo Martins Pereira^{a,*}, Sofia S. Pereira^b, Madalena Santos^b, José Brito^a,
Diamantino Freitas^c, António Oliveira de Carvalho^c, Artur Águas^b, Maria João Oliveira^b,
Pedro Oliveira^a

^a Center for Interdisciplinary Research Egas Moniz (CiEM), Monte da Caparica, Portugal

^b Unidade Multidisciplinar de Investigação Biomédica – UMIB, Universidade do Porto – UP, Porto, Portugal

^c Laboratory of Acoustics, Faculty of Engineering (FEUP), University of Porto, Porto, Portugal



ARTICLE INFO

Keywords:

Infrasound
Glucose intolerance
Liver lipids
Liver steatosis
Rat
Environmental health
Diabetes
Obesity
Hepatobiliary system
Endocrine system
Metabolic disorder
Endocrinology

ABSTRACT

Previous experimental studies show that exposure to noise with high and audible frequencies causes multiple metabolic alterations, such as increased liver glycogen and triglycerides. However, the effect of exposure to sound with lower frequencies, such as high-intensity infrasound (frequency <20 Hz and sound pressure level >90 dB), on the liver lipid content is still unclear. As such, we aimed to study the effect of exposure to high-intensity infrasound of both normal and glucose intolerant rats on the liver lipid content. For this study, 79 wild-type male Wistar rats were randomly divided into two groups: G1, no treatment, and G2, induced glucose intolerance. Each of these two groups was randomly divided in two subgroups: s (animals kept in silence) and i (animals continuously exposed to high-intensity infrasound noise). At three noise-exposure time-points (1, 6 and 12 weeks) the rats were sacrificed, the liver was excised and hepatic lipids extracted. Data analysis was performed using a two-way ANOVA ($p = 0.05$). No significant effects due to interactions between the several factors exist on the liver lipid content ($p = 0.077$). Moreover, no significant effects due to infrasound exposure ($p = 0.407$) or glucose tolerance status ($p = 0.938$) were observed. Our study shows that continuous exposure to high-intensity infrasound has no influence on the lipid content of the liver of both normal and glucose intolerant animals. This finding reinforces the need for further experimental studies on the physiological effects of infrasound due to its possible hazardous effects on human health.

1. Introduction

Noise pollution is an important environmental and occupational risk factor known to cause several adverse effects on human health beyond the auditory system [1]. In Europe, noise was estimated the third environmental risk factor with major impact on public health [2]. The World Health Organization (WHO) Regional Office for Europe has acknowledged that low-frequency noise, below 200 Hz (including infrasound), represents an environmental problem and that research should focus on its outcomes [3].

In previous experimental studies, metabolic abnormalities such as glucose intolerance, insulin resistance, fasting hyperglycemia, dyslipidemia and alterations in insulin signaling in the skeletal muscle have been identified as a consequence of noise exposure with frequencies higher than 200 Hz [4, 5, 6]. Cui et al. [4] also referred increased levels of glycogen and triglycerides in the liver of noise-exposed rats that may lead to non-alcoholic fatty liver disease, a marker of metabolic dysfunction

and risk factor for liver fibrosis, cirrhosis and cancer [7, 8]. This accumulation of lipids in visceral fat is a key player in metabolic derangement and an important risk factor for type 2 diabetes and metabolic syndrome [9]. However, it is still unknown whether exposure to lower frequencies, namely high-intensity infrasound (frequency <20 Hz and sound pressure level >90 dB), induces the same changes in hepatic lipid content.

Therefore, we aimed to investigate if exposure to high-intensity infrasound induces changes in the liver lipids on both normal and glucose intolerant rats and to define the contribution of each of these factors to such outcome.

2. Material and methods

2.1. Animals

Experimental design and planning were performed with full compliance to the PREPARE guidelines [10]. When applied, animal procedures

* Corresponding author.

E-mail address: gpereira@egasmoniz.edu.pt (G. Martins Pereira).

<https://doi.org/10.1016/j.heliyon.2020.e04383>

Received 14 May 2020; Received in revised form 11 June 2020; Accepted 29 June 2020

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were approved by the Portuguese National Authority for Animal Health (project n° 204/2017). All handling and care of the animals were performed by authorized researchers (accredited by FELASA Category C) and was 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 (DL 113/2013).

In compliance with the 3Rs principles [11], this study shares data and resources with a larger study of infrasound-induced pancreatic fibrosis, for which the sample size was estimated based on *a priori* power analysis using G*Power 3 software [12] for a minimum statistical power of 80% (unpublished data). Thus, tissue samples were collected from randomly selected seventy-nine animals of the original sample of one hundred and fifty-six wild-type male Wistar rats acquired from Charles River Laboratories (Saint-Germain-sur-l'Arbresle, France), aged 11 weeks and weighing 375.95 ± 18.29 g. Only male rats were included in order to avoid uncertain sex-dependent differences on the outcomes. They were housed in conventional cages, two animals per cage, with a 12h light/dark cycle (lights on at 8am) and had free access to food (standard rat chow) and water.

After a one-week acclimation period the original sample of one hundred and fifty-six animals were randomly assigned using a free access online software [13] into two groups: G1 (no treatment) and G2 (glucose intolerance). For this study, 39 animals were randomly selected from G1 and 40 animals randomly selected from G2 (Table 1).

2.2. Glucose intolerance

Glucose intolerance was induced through a high-fat diet (D12492 diet, Research Diets) and the administration of low-dose streptozotocin (HFD/STZ rat model) because this model is considered to mimic the human disease [14]. The protocol for glucose intolerance was performed as described by Furman [15]. In short, animals were fed a high-fat diet, with 60% of calories coming from fats, for 3 weeks. After this period STZ (STZ, Sigma) 40 mg/kg was prepared in a sodium citrate buffer 50mM, pH4.4, and was administered intraperitoneally after a fasting period of 6–8 h, with unlimited access to water.

Glucose intolerance was confirmed through an intraperitoneal glucose tolerance test (G2 animals with mean value for glycemia 158.05 mg/dL $\pm$ 30.58 mg/dL at 2h timepoint Vs. G1 animals with mean value for glycemia 123.59 mg/dL $\pm$ 18.39 mg/dL at 2h timepoint, Table 1 and supplemental material) following the protocol established by Ayala et al. [16]. G1 and G2 animals were then fed standard rat chow and were randomly divided in two subgroups each (Table 1): G1s (no treatment, silence, 19 animals), G1i (no treatment, infrasound, 20 animals), G2s (glucose intolerance, silence, 20 animals) and G2i (glucose intolerance, infrasound, 20 animals). Animals from each of the four groups were randomly divided into three infrasound exposure timepoints and euthanized after 1, 6 and 12 weeks of exposure (animals were randomly distributed as stated in Table 1). Before euthanasia, glucose intolerance was again confirmed through an intraperitoneal glucose tolerance test (mean values for glycemia at 2h timepoint and standard deviation for

each experimental group in Table 1 and supplemental material) following the protocol established by Ayala et al. [16].

2.3. Infrasound exposure

Infrasound exposure was performed as previously described by Oliveira et al. [17]. Animal cages were placed in a soundproofed room, measuring $217 \times 211 \times 195$ cm, in front of a noise generator consisting of a subwoofer that reproduced a continuous (24h/day) sound signal, previously recorded in a cotton-mill room from a large textile factory of Northern Portugal. This sound signal was processed offline, applying LabVIEW and Matlab systems.

With the objective of creating a strong subsonic acoustic field in the room, a pseudo-random waveform in the 2-Hz to 20-Hz decade band was filtered from the recorded sound signal 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-Wheavy-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 in the 2–20 Hz with a tolerance of ± 3 dB in a 30 s time window in the entire compartment. 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). Groups not exposed to infrasound were kept in a similar room but in silence.

2.4. Liver lipid content

At the respective timepoint, all rats were euthanized by inhalation of carbon dioxide. Liver was excised and hepatic lipids were extracted according to the protocol established by Folch et al. [18]. Samples of approximately 15mg were obtained and homogenized in a chloroform/methanol solution (v/v, 2:1), shaken for 20 min at room temperature and then centrifuged at 1200 rpm, at 4 °C, for 10 min. Small volumes of 0.9% NaCl were added and centrifuged to separate both phases. The lower phase was evaporated with nitrogen and dried at 100 °C with weighting every 10 min until weight stabilized. Results are expressed as milligrams of lipids per gram of liver.

2.5. Statistical analysis

A univariate general linear model (two-way ANOVA), with dependent variable defined by lipid content and two nominal main factors defined by infrasound exposure and glucose tolerance status, was used for data analysis. The assumptions of normal distribution and variance homogeneity of the lipid content distribution were checked using the Shapiro-

Table 1. Number of animals per experimental group and mean values and standard deviation for glycemia at intraperitoneal glucose tolerance test 2h timepoint, expressed as milligrams per deciliter (mg/dL), for each experimental group of normal (G1) and glucose intolerant (G2) animals, either kept in silence (s) or exposed to high-intensity infrasound (i).

G1 (n = 39)			G2 (n = 40)		
123.59 $\pm$ 18.39			158.05 $\pm$ 30.58		
G1s (n = 19)	1 wk (n = 6)	138.25 $\pm$ 13.77	G2s (n = 20)	1 wk (n = 6)	148.63 $\pm$ 45.54
	6 wks (n = 6)	154.25 $\pm$ 20.45		6 wks (n = 7)	154.75 $\pm$ 17.43
	12 wks (n = 7)	143.75 $\pm$ 21.31		12 wks (n = 7)	168.00 $\pm$ 25.04
G1i (n = 20)	1 wk (n = 6)	156.63 $\pm$ 27.79	G2i (n = 20)	1 wk (n = 6)	151.00 $\pm$ 17.57
	6 wks (n = 7)	142.14 $\pm$ 20.75		6 wks (n = 7)	152.17 $\pm$ 22.34
	12 wks (n = 7)	141.83 $\pm$ 14.23		12 wks (n = 7)	157.00 $\pm$ 11.45

Wilk test and the Levene test, respectively. Data analysis was performed with the software IBM SPSS Statistics for Windows, version 26 (IBM Corp., Armonk, NY, USA), at the 5% significance level ($p = 0.05$).

3. Results

The mean values and standard deviation of lipid content in the liver are illustrated on Table 2 and in Figure 1. The effect of age as a covariable on hepatic lipid content was discarded due to a non-significant Pearson correlation between both variables ($r = 0.137$, $p = 0.228$). Despite this non-significant correlation, the duration of noise exposure was included as covariable in a general linear model with two main factors (high-intensity infrasound exposure and glucose tolerance status), after validation of the assumption of homogeneity of variance (Levene test, $p = 0.460$). It should be noted that the assumption of normal distribution of lipid content hold, except in one subgroup (Shapiro-Wilk test, $p = 0.026$), in which however no severe symmetry was detected. Furthermore, no significant interaction between the covariate and the main factors included in the model was observed. The results show that no significant effects due to interactions between the several factors exist on the liver lipid content ($p = 0.077$). Moreover, no significant effects due to high-intensity infrasound exposure ($p = 0.407$) or glucose tolerance status ($p = 0.938$) were observed.

However, and despite not being statistically significant ($p = 0.077$), our results may suggest the existence of an interaction between factors, that is, that the response to noise exposure with regard to the hepatic fat content depends on the metabolic condition of the animals, which is also suggested by Figure 2. Nevertheless, these conclusions must be considered with caution, in light of the above.

4. Discussion

Our study aimed to investigate whether chronic exposure to high-intensity infrasound could trigger metabolic changes in the liver, namely on its lipid content, in both normal and glucose intolerant rats. Our results show that there is no influence from such exposure on this outcome, although in glucose intolerant rats the liver lipid content is slightly increased, which may be due to chance, but also reinforces the need of further evaluations to address if the presence of glucose intolerance may be an additional risk factor for the alterations induced by chronic exposure to high-intensity infrasound.

High-intensity infrasound exposure studies in laboratory animals addressing liver lipid content are scarce since most studies focus on audible noise with higher frequencies [4, 5, 6]. In these studies, an increase in hepatic concentration of glycogen and triglycerides has been described [4]. Several theoretical models for the association between audible noise exposure and metabolic changes have been developed,

focusing on the role of noise as a stressor, and as a trigger of the neuroendocrine pathways that promote hyperglycemia, insulin resistance and fat accumulation [19, 20]. Liver steatosis can also result from endoplasmic reticulum stress, through impaired fatty acid oxidation and disturbance of the unfolded protein response [21].

Previous experimental studies support the role of audible noise exposure as a liver stressor [22, 23]. These studies have demonstrated that chronic stress associated with an enriched diet increases the levels of total cholesterol and triglycerides in the liver, as well as hepatic inflammation and oxidative stress [22], aggravating the induced non-alcoholic fatty liver disease from steatosis to steatohepatitis [23].

Infrasound is a mechanical vibration wave with a frequency range below 20 Hz, originated by natural phenomena and man-made sources, such as industrial installations, low-speed machinery and music [24, 25]. Due to its wavelength, infrasound can propagate over very large distances without being reflected or absorbed by obstacles and is hardly attenuated through dissipation [24]. As such, infrasound can induce body vibrations and resonance in body cavities, thus affecting internal systems and organs [26].

There is evidence from high-intensity infrasound exposure studies in laboratory animals that chronic exposure results in proliferation of the connective tissue matrix and collagen fibers in animals; this fibrotic response has been documented in several organs, such as the heart, lung and glands of rats chronically exposed to industrial-type noise [27, 28, 29, 30]. Oliveira et al. [31] documented the same alterations in the liver connective tissue, on centrilobular regions without disruption of the organ architecture, as a result of the exposure to high-intensity infrasound. This is thought to be a response to the body vibrations induced by infrasound and may function as a mechanical stabilizer of the organ [30].

However, there is a common misconception about the inaudibility of infrasound since sounds with lower frequencies can still be heard with an increase of the sound pressure level [24, 26]. Higher pressure levels, as the ones used in our study, can elicit both body vibration and hearing response from the animal model used [32, 33]. As such, we cannot exclude animal stress due to audible noise with subsequent activation of the neuroendocrine pathways. To answer this question, future studies should assess clinical and behavioral signs along with corticosterone, the primary stress hormone in rodents, to examine the stress response of the experimental animal [34].

The major limitation of our study is the number of animals, as the study sample was drawn from an original sample estimated for a larger metabolic experimental study on infrasound-induced pancreatic fibrosis (according to the 3Rs principles), as stated in section 2.1 [11]. On the other hand, the experimental protocol allows the assessment of interactions between other important variables studied. We also considered the effect of aging on our experimental protocol, since lipogenesis

Table 2. Mean values and standard deviation for liver lipid content, expressed as milligrams of lipids per gram of liver in normal (G1) and glucose intolerant (G2) animals, either kept in silence (s) or exposed to high-intensity infrasound (i) at different timepoints. No significant effects on the liver lipid content were observed, due to interactions between factors ($p = 0.077$), infrasound exposure ($p = 0.407$) or glucose tolerance status ($p = 0.938$).

Group		Timepoint of sacrifice	Liver Lipid Content
No treatment (G1)	G1s	week 1	54.78 (± 7.91)
		week 6	49.00 (± 8.23)
		week 12	59.00 (± 8.58)
	G1i	week 1	54.13 (± 19.91)
		week 6	49.50 (± 5.65)
		week 12	51.71 (± 9.52)
Glucose intolerance (G2)	G2s	week 1	51.20 (± 3.49)
		week 6	48.33 (± 4.89)
		week 12	50.40 (± 11.06)
	G2i	week 1	48.00 (± 17.09)
		week 6	56.20 (± 10.64)
		week 12	61.00 (± 4.65)

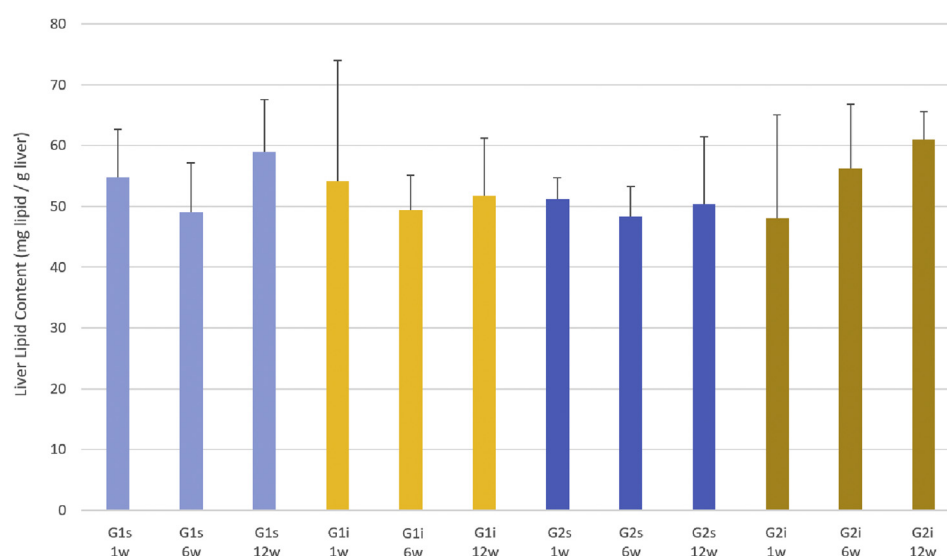


Figure 1. Liver lipid content of normal (G1) and glucose intolerant rats (G2) kept in silence (s) and exposed to high-intensity infrasound (i) at different timepoints. A non-significant effect on the liver lipid content due to interactions between factors ($p = 0.077$) was observed, as well as due to glucose tolerance status ($p = 0.938$) or noise exposure ($p = 0.407$).

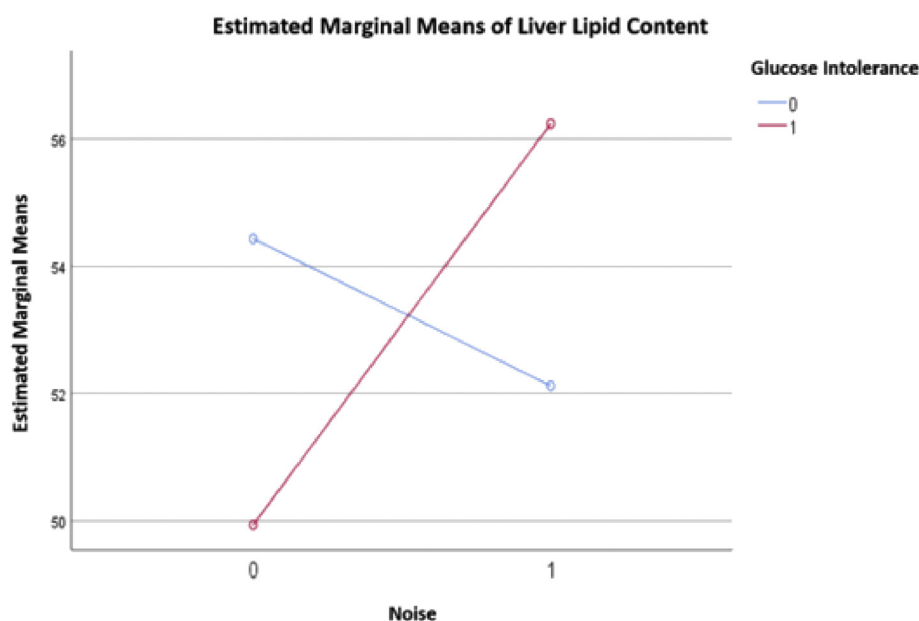


Figure 2. Comparison of estimated marginal mean values of liver lipid content in relation with noise exposure and glucose tolerance status. No significant effects were observed on the liver lipid content due to interactions between factors ($p = 0.077$).

and fat accumulation leading to liver steatosis are both part of the natural aging process of the liver [35, 36]. Accordingly, we used age-matched animals, as control groups, and the effect of time as a covariable was discarded due to a non-significant correlation between both variables. We have found a discrete, non-significant increase, of liver lipid in the glucose intolerant rats that may be due to chance. Nevertheless, and although we had 79 animals, future studies should consider the possibility of this additional risk factor for the alterations induced by chronic exposure to high-intensity infrasound.

In summary, our study shows that continuous exposure to high-intensity infrasound has no influence on the liver lipid content of both normal and glucose intolerant animals. Within the limitations of our study, these results reinforce the importance of further research concerning the effects of high-intensity infrasound, a ubiquitous element, on the liver due to its possible hazardous effects on human health.

Declarations

Author contribution statement

Gonalo Martins Pereira: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Sofia Pereira, Madalena Santos: Conceived and designed the experiments; Performed the experiments; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Jos  Brito: Analyzed and interpreted the data; Wrote the paper. Diamantino Freitas, Ant nio Carvalho: Conceived and designed the experiments; Performed the experiments; Wrote the paper.

Artur  guas: Conceived and designed the experiments; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Maria João Oliveira, Pedro Oliveira: Conceived and designed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Funding statement

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Competing interest statement

The authors declare no conflict of interest.

Additional information

Supplementary content related to this article has been published online at <https://doi.org/10.1016/j.heliyon.2020.e04383>.

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