Published in final edited form as:

J Ultrasound Med. 2017 April ; 36(4): 799–808. doi:10.7863/ultra.16.02017.

The negative chronotropic effect in rat heart stimulated by ultrasonic pulses: role of sex and age

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Abstract

Objectives—The goal of this study is to investigate the role of sex and age of the negative chronotropic effect after exposure of 3.5-MHz pulsed ultrasound (US) to the rat heart.

Methods—Forty F344 rats were exposed transthoracically to ultrasonic pulses at a duty factor of ~1.0% at 2.0-MPa peak rarefactional pressure amplitude (PRPA). The transthoracic ultrasonic bursts were delivered consecutively in five 10-s intervals, that is, 10 s of 6-Hz pulse repetition frequency (PRF), 10 s of 5-Hz PRF, 10 s of 4-Hz PRF, 10 s of 5-Hz PRF and 10 s of 6-Hz, for a 50-s total exposure duration. The rats were divided into eight groups (n=5 ea) animals: US young male, Control young male, US young female, Control young female, US old male, Control old male, US old female and Control old female.

Results—Two-way ANOVA for repeated measures was used to compare heart rate, cardiac output, arterial pressure and other hemodynamic values (baseline) before and after ultrasound stimulation. Sex vs. age vs. US interaction was detected for heart rate. Cardiac output showed an age effect and ejection fraction showed age and US effects. The arterial pressure showed a sex effect. A negative chronotropic effect (~30% decrease in heart rate) was observed for young female rats. A hypothesis is that the US effect is weight (menopause)-dependent, because the young (premenopause) female rats weighed ~40–60% less than other groups of rats.

Conclusion—It is likely that the ovarian hormones are responsible for different ultrasound-induced cardiac bioeffects in different ages and sexes.

Keywords

sex; age; ultrasound; biological effects; heart; chronotropic effect

I. INTRODUCTION

Gender and sex differences are increasingly being recognized in medicine and especially in cardiology.¹ Cardiovascular disease remains the primary cause of death worldwide,

especially in the United States where deaths due to cardiovascular disease in women exceed those of men. Cultural factors as well as physiological differences between women and men can contribute to development of cardiovascular disease.² However, the incidence and the progressive rate of cardiovascular disease are markedly higher in men when age-matched with premenopausal women.³ Women exhibit a delay in the onset of vascular disease compared to men and the temporal link between menopause and the rise in vascular events in women suggests that ovarian hormones may be important in reducing the risk of vascular disease in women.⁴ Clinical studies have shown that women who have had a hysterectomy have a higher prevalence and incidence of cardiovascular disease and hypertension (irrespective of age when the hysterectomy was performed), indicating that the lack of ovarian hormones is associated with a greater rate of cardiovascular disease.⁵ Animal models for cardiovascular disease support the hypothesis that female sex and/or the sex hormone estrogen may contribute to the sexual dimorphism in the heart and to a better outcome of cardiac diseases in females.⁶

The study of cardiac pacing using pulsed ultrasound is clinically important as an alternative and leadless source of energy. All pacing leads are associated with complications such as infection, fracture, and dislodgment, so there is a clinical need to develop a pacing system that reduces the problems inherent with the pacing leads.^{7,8} Additionally, the extraction of a failed lead that has been implanted for a long time is likely to be a high-risk procedure, including serious arterial injuries or even death.^{9,10,11} Women have had, independent of age or implanted pacing systems, significantly more acute complications than men, with significant differences for pneumothorax and pocket haematoma.¹ The incidence of arrhythmias in women is also higher, possibly due to gender-specific variations in the electrophysiological structure of the heart or hormonal effects.¹²

Elevated heart rate is associated with increased peripheral blood pressure, increased risk for cardiovascular disease, and is modifiable. However, in the absence of heart failure or coronary artery disease, treating hypertensive patients with heart rate lowering medications has not been shown to reduce adverse events when compared to active control.¹³ Previous studies in rat hearts demonstrated that transthoracic ultrasound can promote a negative chronotropic effect without impairing the cardiac pumping function.^{14,15,16,17} This negative chronotropic effect was achieved by means of a specific sequence of ultrasonic bursts delivered to the heart, progressively decreasing the pulse repetition frequency.

Pulsed ultrasound has been shown to interfere in the cardiac activity of the turtle,¹⁸ dog,¹⁹ frog,²⁰ mouse,²¹ pg,²² guinea pig²³ and rat.^{14,15,16,17,24} Animal models used for identifying sex-related differences have been developed for different kinds of research, including atherosclerosis, toxicology, brain injury, stress/alcohol consumption, autoimmune diseases and hormones.²⁵ However, it is not known whether an animal model has been developed for ultrasound/cardiac pacing/therapy for different sexes and ages. The aim of the study is to evaluate whether sex differences play a role in cardiac therapy, evaluating the feasibility and biophysics of a technology that uses pulsed ultrasound to achieve leadless cardiac stimulation without adversely affecting cardiac tissue.

A more complete study of ultrasound-induced cardiac risk must take into account “premenopause” and “postmenopause” in the animal model that, in the case of the rat, is estimated in terms of fertility. Female rat fertility begins to subside between 15 and 18 months of age. Thus, an age component was built into the study.

The purpose of this study was to investigate the role of sex and age (along with ultrasound) on the negative chronotropic effect. These outcomes would provide further evidence/insight for determining the mechanism that involves the decrease of the rat's heart rate as a function of different sexes and ages and, further, interfere possible therapeutic applications of controlled ultrasonic pulses in cardiology.

II. MATERIALS AND METHODS

Animals

Experimental conditions were approved by the University of Illinois Institutional Animal Care and Use Committee (protocol #10104). Forty F344 rats (Harlan, Indianapolis, IN) were divided into eight groups (n = 5 ea): groups 1 & 2) three-mo-old 150–200 g 1) US young female and 2) Control young female; groups 3 & 4) three-mo-old 300–350 g 3) US young male and 4) Control young male; groups 5 & 6) 24-mo-old 250–300 g 5) US old female and 6) Control old female and groups 7 & 8) 24-mo-old 400–500 g 7) US old male and 8) Control old male. Animals received 5% isoflurane for inhalation anesthesia induction, and then 1.5–2% isoflurane for anesthesia maintenance. The level of anesthesia was monitored by pedal reflex. The thoracic region was shaved and depilated to maximize transthoracic acoustic transmission. Gel was used for acoustic coupling. Rats were placed on a temperature-controlled platform in dorsal recumbency for transthoracic US exposure of the heart. The animal was placed in dorsal recumbency to allow the measurement of the arterial pressure through the femoral artery and the ultrasound application simultaneously. It is unlikely that this position would affect the heart rate or arterial pressure. Dorsal recumbency can compromise ventilation and O₂ transport but the respiratory rate did not change significantly before and after the study.

The animals' limbs were secured to the EKG pads (4 leads) on the animal platform, a capability integral with the Vevo2100 high-frequency ultrasound imaging system (VisualSonics, Toronto, ON, CA), so that such physiological data could be monitored in real time and also recorded.

In physiologic and cardiologic studies, it is often the practice to record arterial pressure from an easily accessible artery (e.g., femoral, tail or carotid artery), and to use that pressure to represent the systemic arterial pressure or mean pressure.¹⁰ In this study arterial pressure was monitored to assess possible changes that might elicit reflex responses that affect the heart. The left femoral artery of all rats was cannulated for arterial pressure measures. The cannula was connected to a pressure transducer (Samba Preclin 420LP, Samba Sensors, Gothenburg, Sweden) and a control unit (Samba 201, Samba Sensor, Gothenburg, Sweden). Also, the rectal temperature was monitored in all rats. The animal's leg region remained opened for the balance of the experimental procedures.

For all rats, arterial pressure, rectal temperature and EKG were recorded continuously from prior to the initiation of the US application until about 15 min after the US exposure ceased (see Fig 1). Then (≈ 18 min), rats were euthanized (CO_2 inhalation for 5 min), and the lung and heart were removed, fixed, and processed for evaluation by a Board-certified pathologist.

Ultrasound

An unfocused 19-mm-diameter aperture 3.5-MHz ultrasonic transducer was used (Valpey Fisher, Valpey Corporation, Hopkinton, MA). The transducer was calibrated in a tank of distilled, degassed 22°C water. The calibrated hydrophone was a polyvinylidene fluoride (PVDF) membrane hydrophone (Y-34–3598 EW295, GEC Marconi, Chelmsford, UK) with a 0.5-mm-diameter active element. The transducer was held in a fixed position while the hydrophone was moved perpendicular to the beam axis at a distance of 1 cm from the transducer surface (in the near field) by a micropositioning system ($2\text{-}\mu\text{m}$ translational accuracy).¹²

The transducer was driven by a function generator (33250A, Agilent Technologies Inc., Santa Clara, CA) and an RF (radio frequency) power amplifier (A500, Electronic Navigation Industries, Rochester, NY; 0.3 to 35 MHz; 55 dB). The Vevo2100 ultrasound imaging system, operated by a registered diagnostic medical sonographer (RDMS), was used to monitor the heart via B-mode and M-mode displays. The Vevo2100 workstation has a tool to calculate the ejection fraction and other cardiac parameters by ventricular trace. In order to minimize thermal effects,¹⁵ the following US protocol was applied (Fig 1): US bursts of 2.0-MPa PRPA (equivalent to an in vitro spatial peak temporal peak intensity of $\sim 270\text{ W/cm}^2$ and a Mechanical Index of 1.1) were delivered consecutively in five 10-s intervals, that is, 10 s for each PRF (6, 5, 4, 5 and 6 Hz) for a 50-s exposure duration. The PRF sequence started slightly above the heart rate of the rat (for rats the heart rate is $\sim 300\text{--}350$ beats per minute or 5 Hz), decreasing and increasing 1 Hz gap. The duty factor was approximately 1.0%, which means that for a PRF sequence of 6, 5, 4, 5 and 6 Hz (i.e., 167, 200, 250, 200 and 167-ms pulse repetition periods, correspondingly), a 2-ms pulse duration was used.

The in situ (in/at the heart) exposure parameters are estimated from a relatively complete experimental study to chest wall attenuation and thickness for the Sprague-Dawley rats²⁶ for which the attenuation at 3.5 MHz was $\sim 1.2\text{ dB/cm-MHz}$ and the thickness was $\sim 3.6\text{ mm}$. Thus, the in situ ultrasonic pressure is $0.84 \times 2.0\text{ MPa} = 1.7\text{ MPa}$ (Fig. 2) and the in situ ultrasonic spatial peak temporal peak intensity is $0.70 \times 270\text{ W/cm}^2 = 190\text{ W/cm}^2$. Using the previously published procedures¹⁵ with these in situ exposure parameters, the maximum in situ temperature increase from a single 2-ms-duration pulse is 0.009°C . With the $\sim 1\%$ pulse duration, the steady-state in situ temperature increase is estimated to be 0.89°C , assuming to heat removal processes.

Statistical Analysis

The data were organized in a standard $2\text{ (sex)} \times 2\text{ (age)} \times 2\text{ (US)}$ factorial design with sex (male versus female) as one factor, age (young versus old) as another factor and ultrasound (on versus off), see Fig 3. Two-way ANOVA (R version 3.0.2, “Frisbee Sailing”, The R

Foundation for Statistical Computing, Auckland, New Zealand) for repeated measures was performed between sex, age and US at 3 and 15 min post US exposure compared to baseline for the variables: heart rate, cardiac output, systolic volume, ejection fraction, end-diastolic volume, end-systolic volume, respiratory rate and arterial pressure. Bonferroni *post hoc* tests were applied to compare the results. All results were expressed as mean and standard error of the mean (SEM). The significance level was set at 0.05.

III. RESULTS

The absolute values of physiological parameters at baseline and at 3 and 15 min post US exposures are listed for young male rats (Table 1), young female rats (Table 2), old male rats (Table 3), and old female rats (Table 4). Table 5 displays a summary of the statistical analysis outcomes for that physiological variable that yielded statistically significant results.

Baseline

There were no significant interactions between sex and age for heart rate, cardiac output, systolic volume, ejection fraction, end-diastolic volume, end-systolic volume, respiratory rate and arterial pressure.

Heart Rate

At 3 min post US exposure, the ultrasound effect (decrease of the heart rate) was significant ($p<0.001$), the age effect was significant ($p<0.01$) and the sex effect was not significant. The interactions between sex, age and ultrasound were significant ($p<0.001$). At 15 min post US exposure, the ultrasound effect (decrease of the heart rate) was significant ($p<0.01$), the sex and age effects were not significant and there were no significant interactions (Fig. 4-A). The Bonferroni test showed a significant effect at 3 min post US exposure between age (young vs. old) and US (on vs. off). And, at 15 min post US exposure the Bonferroni test showed a significant effect between sex (male vs. female), age (young vs. old) and US (on vs. off).

Cardiac Output

At 3 min post US exposure, the ultrasound and sex effects were not significant, and the age effect was significant ($p=0.03$). There were no significant interactions. At 15 min post US exposure, there were no significant effects or interactions (Fig. 4-B). The Bonferroni test showed a significant effect at 3 min post US exposure between sex (male vs. female) and age (young vs. old).

Ejection Fraction

At 3 min post US exposure, the ultrasound effect was significant ($p<0.001$), the age effect was significant ($p<0.01$) and the sex effect was not significant. There were significant interactions between sex, age and US ($p<0.001$). At 15 min post US exposure, there were significant US ($p<0.001$) and sex ($p=0.03$) effects but no significant interactions (Fig. 4-C). The Bonferroni test showed a significant effect at 3 and 15 min post US exposures between US (on vs. off).

For systolic volume, end-diastolic volume (Fig. 4-D) and respiratory rate at both 3 and 15 min post US exposure, there were no significant effects or interactions.

End-systolic volume

At 3 min post US exposure, the ultrasound effect was significant ($p<0.001$). The interactions between sex and age and ultrasound were significant ($p<0.01$). At 15 min post US exposure, there was a significant US effect ($p<0.001$), and no significant sex or age effects or interactions (Fig. 4-E). The Bonferroni test showed a significant effect at both 3 and 15 min post US exposure between sex (male vs. female) and US (on vs. off).

Arterial Pressure

At 3 min post US exposure, there was a significant sex effect ($p=0.01$). There was no significant age or US effects or interactions (Fig. 4-F). At 15 min post US exposure, there were no significant effects or interactions. The Bonferroni test showed no differences between age, sex and US.

Histological examination of the hearts and lungs of all animals exposed to ultrasound did not show lesions after the procedure.

IV. DISCUSSION

The goal of this study was to investigate the role of the sex and age on the ultrasound-mediated depression of the heart rate. There were no differences between sex and age at baseline. For heart rate at 3 and 15 min post US exposure, there were ultrasound effects but no sex effects; there were age and interaction effects at 3 min post US exposure but not at 15 min post exposure. The cardiac output results showed an age effect at 3 min post US exposure. At 3 min post US exposure, the ejection fraction showed age and ultrasound effects. And, at 15 min there was a sex and US effect. For the end-systolic volume there was an ultrasound effect at 3 and 15 min post US exposure. There were no effects or interactions for stroke volume, end-diastolic volume and respiratory rate.

Table 5 shows that significant sex effects were observed for ejection fraction at 15 min post US exposure and arterial pressure at 3 min post US exposure. For age, there were significant effects for heart rate, cardiac output and ejection fraction only at 3 min post US exposure. It is unlikely that sex and age plays a role in the decrease of the heart rate. The common effect was caused by ultrasound application and it was significant for both 3 and 15 min post US exposure.

Also, Table 5 shows for heart rate, ejection fraction and end-systolic volume that there was a significant interaction between sex vs. age vs. US. The negative chronotropic effect depends of sex, age and ultrasound for heart rate, ejection fraction and end-systolic volume.

However, the Bonferroni test showed a significant effect at 3 min post US exposure between age (young vs. old) and US (on vs. off), and, at 15 min post US exposure the Bonferroni test showed a significant effect between sex (male vs. female), age (young vs. old) and US (on vs. off). In this case, the variable sex does not play a role in the decrease of the heart rate. For ejection fraction, the Bonferroni test showed a significant effect at 3 and 15 min post US

exposures between US (on vs. off) and interaction between sex, age and US, suggesting that the effect was due to ultrasound. The Bonferroni test showed a significant effect at both 3 and 15 min post US exposure between sex (male vs. female) and US (on vs. off) for the end-systolic volume and no significant effect for sex itself. This result suggests that US is playing a role instead of age.

Additional studies need to be conducted to examine the parasympathetic system through heart rate variability analysis and vagal efferent activity. As such investigate the sympathetic withdrawal doing peripheral receptor blockade or chemical sympathectomy. It is possible that some changes in the protocol need to occur to understand the mechanism of the decrease of the heart rate after ultrasound application and some limitations might raise from this investigation.

In this study a decrease in the heart rate (~30%) was observed in the young female rats at 3 min post US exposure (Fig. 4-A). The US exposure parameters caused a cardiac output depression of ~37% and an ejection fraction decrease of ~18% at 3 min post US exposure. The end-systolic volume increased ~150% at 3 min post US exposure. As the decrease of heart rate increases the duration of diastole, ventricular filling / end diastolic volume is expected to increase. In young females the end-diastolic volume was increased (~5%) (Frank-Starling mechanism). Contraction of cardiac muscle is influenced by both preload and afterload. Higher systolic pressures can be reached during ventricular contractions by raising afterload. Incremental increases in afterload produce progressively higher peak systolic pressures. It seems that the US application in young female rats caused a change in the cardiac contractility that was not observed in the other animals. One hypothesis is that the US effect is weight-dependent; the young female rats compared with the young males and old females weighed ~40% less, and comparing with old males weighed ~60% less. Also, the size of the heart in the young females was smaller than the other rats (typical ranges are 1.5–2.5 cm external length and 1.3–1.5 cm external width).

As the same US transducer was used (1.9-cm-diameter beamwidth unchanged because the heart is within the first 2 cm (10%) of the Fresnel zone; Fresnel zone ~20 cm for the 1.9-cm-diameter 3.5-MHz transducer), it is always possible that the US field interact with more cardiac structures in the smaller rats than the larger rats, thus causing a change in the contractility of the heart, but is not too likely.

A second hypothesis is the hormones respond different between the sexes. Differences in animals models support the idea that sex hormones and their steroid hormone receptors regulate this cardiovascular response.²⁵ A few studies focused in cardiac hypertrophy showed a significant increase in cardiac mass in female rats²⁷ and female mice^{28,29,30} better contractile function in male rats³¹ and improved myocardial function in female hearts.³² In this study the young female rats showed a marked negative chronotropic effect; it is important to note that their estrogen levels are much higher than the older “postmenopause” rats. It appears that the hormone contributions are critical in the observed outcomes.

In previous study,¹⁷ the intrathoracic (intercostal space) and rectal temperatures were monitored and no significant changes were observed after ultrasound exposure. Since the

intrathoracic temperature measure is invasive and previous results did not show any changes, herein only the rectal temperature was monitored. Histological examination of the heart and lungs of all animals exposed to ultrasound did not show any gross abnormalities following the ultrasonic exposure procedures.

It is not clear what mechanism is involved in the negative chronotropic effect. In a previous study,¹⁶ it was proposed that the negative chronotropic effect resulting from the ultrasound application may have occurred from parasympathetic stimulation. In this study the ultrasound-induced risk to the heart must take into account “premenopause” and “postmenopause” in the animal model. However, the study showed a difference between age and sex effects, with different outcomes for male and female rats. A significant difference was observed on the heart rates of old female rats versus young female rats, as was also the case for young male rats versus young female rats.

Another possibility showed in previous study¹⁶ was that the direct ultrasound stimulation of aortic baroreceptors could trigger the baroreceptor reflex, whose output translates into an increased parasympathetic tone and decreased sympathetic tone with consequent bradycardia. In this study the outcomes for arterial pressure showed a difference between sexes. A decrease in the arterial pressure of ~23% was observed for young female rats at 3 min post US exposure. With the decrease of the cardiac output (heart rate x stroke volume) and the heart rate, the arterial pressure must decrease. This outcome suggests that the negative chronotropic effect could be caused by indirect baroreceptor stimulation from US application.

The pulsed ultrasonic exposure caused a negative chronotropic effect in the young female rat heart. The statistical analysis showed interaction between sex, age and ultrasound. Age and sex differences appear to play a role in the decrease of the heart rate in rats. The results suggest that ovarian hormones might be a cause for the different heart-related bioeffects. These results raise the possibility of circulatory depression resulting from therapeutic ultrasonic stimulation, and stress the need of additional studies to elucidate the physiological mechanisms involved in the production of these effects.

Acknowledgments

This work was supported by the National Institutes of Health (R37 EB002641). The authors would like to acknowledge the technical assistance by Rita J. Miller, DVM, Emily Hartman, BS, RDMS and Sandhya Sarwate, MD.

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Figure 1.

Time line for the 3.5-MHz transthoracic high-amplitude ultrasound stimulation. Baseline data were acquired prior to the initial ultrasound exposure. Post-exposure data were acquired at 3 min and 15 min after ultrasound ceased. The ultrasonic exposure consisted of one sequence of 10 s each (each with a different PRF). The total duration of the stimulation protocol was 50 s. Duty factor (DF) is approximate because the pulse duration was 2 ms.

Figure 2.
Transducer pressure response at ~2 MPa.

	Male	Female
old	Ultrasound off Old Male	Ultrasound off Old Female
	Ultrasound on Old Male	Ultrasound on Old Female
young	Ultrasound on Young Male	Ultrasound on Young Female
	Ultrasound on Young Male	Ultrasound on Young Female

Figure 3.

$2 \times 2 \times 2$ factorial design with sex (male versus female) as on factor, age (young versus old) as another factor and ultrasound (on versus off).

Figure 4. Normalized values of; A) heart rate, B) cardiac output, C) ejection fraction, D) end-diastolic volume, E) end-systolic volume and F) arterial pressure at 3 min (right side) and 15 min (left side) after US application for all 8 groups.

Absolute values of physiological parameters at baseline and at 3 and 15 min post US exposures (Fig 1) for US young male and Control young male groups. All values are expressed as mean and standard error of the mean (SEM). There were no significant interactions between sex and age for heart rate, cardiac output, systolic volume, ejection fraction, end-diastolic volume, end-systolic volume, respiratory rate and arterial pressure.

Table 1

PARAMETER	Control young male (n=5)				US young male (n=5)			
	Baseline	3 min post US	15 min post US	Baseline	3 min post US	15 min post US	Baseline	15 min post US
Heart Rate (BPM)	285.20 ± 27.29	273.6 ± 28.63	270.00 ± 28.45	246.40 ± 13.30	233.00 ± 8.62	229.80 ± 10.62		
Cardiac Output (mL/min)	43.49 ± 2.96	40.62 ± 1.46	40.70 ± 2.70	41.37 ± 3.73	39.51 ± 2.85	41.55 ± 3.25		
Systolic volume (μL)	156.08 ± 9.69	155.75 ± 15.55	172.56 ± 14.54	168.33 ± 13.76	169.98 ± 12.27	180.82 ± 11.23		
Ejection Fraction (%)	87.06 ± 4.40	87.57 ± 3.24	86.68 ± 2.89	82.38 ± 2.46	80.37 ± 2.03	81.41 ± 1.60		
End-diastolic volume (μL)	185.43 ± 20.36	182.24 ± 25.32	201.21 ± 20.45	202.33 ± 12.11	210.65 ± 11.37	221.71 ± 12.08		
End-systolic volume (μL)	29.35 ± 12.11	26.49 ± 10.00	28.65 ± 7.93	35.80 ± 4.96	40.68 ± 3.19	40.89 ± 3.41		
Respiratory rate (min ⁻¹)	41.20 ± 3.12	42.80 ± 3.02	43.00 ± 3.11	37.20 ± 2.46	35.40 ± 1.73	36.40 ± 2.05		
Arterial pressure (mmHg)	86.80 ± 2.95	87.60 ± 4.50	84.40 ± 4.20	95.20 ± 1.30	98.00 ± 2.77	95.60 ± 2.86		

Absolute values of physiological parameters at baseline and at 3 and 15 min post US exposures (Fig 1) for US young female and Control young female groups. All values are expressed as mean and standard error of the mean (SEM). There were no significant interactions between sex and age for heart rate, cardiac output, systolic volume, ejection fraction, end-diastolic volume, end-systolic volume, respiratory rate and arterial pressure.

Table 2

PARAMETER	Control young female (n=5)				US young female (n=5)			
	Baseline	3 min post US	15 min post US	Baseline	Baseline	3 min post US	15 min post US	Baseline
Heart Rate (BPM)	318.60 ± 4.46	311.60 ± 4.57	302.00 ± 4.32	306.20 ± 17.86	315.80 ± 11.00	315.80 ± 11.00	251.60 ± 4.92	315.80 ± 11.00
Cardiac Output (mL/min)	33.82 ± 1.54	36.41 ± 2.22	35.06 ± 2.00	30.61 ± 3.05	19.44 ± 2.48	19.44 ± 2.48	26.08 ± 2.08	19.44 ± 2.48
Systolic volume (μL)	106.12 ± 4.72	117.37 ± 8.66	115.99 ± 7.04	102.19 ± 6.24	94.07 ± 6.03	94.07 ± 6.03	110.50 ± 7.86	94.07 ± 6.03
Ejection Fraction (%)	85.89 ± 1.38	90.47 ± 0.89	90.00 ± 0.79	85.42 ± 2.97	70.03 ± 3.77	70.03 ± 3.77	81.81 ± 2.71	70.03 ± 3.77
End-diastolic volume (μL)	123.35 ± 3.95	129.82 ± 9.80	128.71 ± 7.14	116.50 ± 7.00	126.68 ± 9.03	126.68 ± 9.03	126.75 ± 8.60	126.68 ± 9.03
End-systolic volume (μL)	17.23 ± 1.73	12.45 ± 1.62	12.72 ± 0.82	16.75 ± 3.39	37.97 ± 5.62	37.97 ± 5.62	23.15 ± 3.72	37.97 ± 5.62
Respiratory rate (min ⁻¹)	44.60 ± 3.27	44.20 ± 3.33	45.00 ± 2.94	41.40 ± 2.31	38.00 ± 4.11	38.00 ± 4.11	39.60 ± 1.31	38.00 ± 4.11
Arterial pressure (mmHg)	94.40 ± 7.16	92.00 ± 9.26	93.00 ± 8.73	74.20 ± 3.27	56.20 ± 8.26	56.20 ± 8.26	64.00 ± 8.69	56.20 ± 8.26

Table 3

Absolute values of physiological parameters at baseline and at 3 and 15 min post US exposures (Fig 1) for US old male and Control old male groups. All values are expressed as mean and standard error of the mean (SEM). There were no significant interactions between sex and age for heart rate, cardiac output, systolic volume, ejection fraction, end-diastolic volume, end-systolic volume, respiratory rate and arterial pressure.

PARAMETER	Control old male (n=5)			US old male (n=5)		
	Baseline	3 min post US	15 min post US	Baseline	3 min post US	15 min post US
Heart Rate (BPM)	231.40 ± 6.08	224.00 ± 6.10	218.20 ± 6.68	220.80 ± 6.15	217.00 ± 5.00	210.40 ± 4.95
Cardiac Output (mL/min)	54.54 ± 3.13	48.28 ± 4.46	49.74 ± 4.01	55.70 ± 4.53	52.10 ± 6.61	53.16 ± 4.23
Systolic volume (μL)	222.30 ± 6.82	206.54 ± 13.82	213.94 ± 11.05	253.06 ± 20.42	238.45 ± 27.81	251.84 ± 17.67
Ejection Fraction (%)	84.41 ± 1.50	85.26 ± 1.58	85.21 ± 1.38	78.36 ± 2.64	75.42 ± 2.93	77.03 ± 2.61
End-diastolic volume (μL)	263.84 ± 9.66	241.40 ± 13.56	250.83 ± 11.83	323.05 ± 23.64	315.64 ± 33.31	328.11 ± 21.65
End-systolic volume (μL)	41.54 ± 4.64	34.86 ± 2.79	36.89 ± 3.49	69.99 ± 10.75	77.19 ± 12.30	75.67 ± 10.89
Respiratory rate (min ⁻¹)	42.40 ± 2.38	40.80 ± 2.50	38.00 ± 2.46	43.80 ± 3.47	43.20 ± 4.19	43.80 ± 4.77
Arterial pressure (mmHg)	67.60 ± 2.49	71.60 ± 1.95	69.80 ± 1.84	83.00 ± 7.77	80.40 ± 5.35	80.00 ± 5.40

Table 4

Absolute values of physiological parameters at baseline and at 3 and 15 min post US exposures (Fig 1) for US old female and Control old female groups. All values are expressed as mean and standard error of the mean (SEM). There were no significant interactions between sex and age for heart rate, cardiac output, systolic volume, ejection fraction, end-diastolic volume, end-systolic volume, respiratory rate and arterial pressure.

PARAMETER	Control old female (n=5)			US old female (n=5)		
	Baseline	3 min post US	15 min post US	Baseline	3 min post US	15 min post US
Heart Rate (BPM)	233.80 ± 11.84	244.00 ± 9.74	249.00 ± 9.81	240.60 ± 11.16	241.40 ± 9.88	254.62 ± 18.86
Cardiac Output (mL/min)	36.21 ± 4.03	43.09 ± 4.64	43.67 ± 4.74	34.14 ± 2.61	37.13 ± 2.21	35.00 ± 0.81
Systolic volume (μL)	136.61 ± 16.84	164.50 ± 10.42	164.59 ± 10.29	143.41 ± 11.92	154.36 ± 9.40	149.05 ± 5.74
Ejection Fraction (%)	84.18 ± 2.65	88.77 ± 0.97	89.57 ± 0.91	83.42 ± 2.95	85.20 ± 2.06	84.55 ± 1.69
End-diastolic volume (μL)	160.93 ± 16.45	185.12 ± 10.82	183.85 ± 11.53	172.30 ± 13.94	181.13 ± 9.79	176.70 ± 7.92
End-systolic volume (μL)	24.33 ± 4.11	20.63 ± 1.84	19.26 ± 2.24	28.88 ± 6.18	26.76 ± 3.75	27.64 ± 3.77
Respiratory rate (min ⁻¹)	42.40 ± 3.23	45.60 ± 2.60	45.20 ± 2.98	37.20 ± 2.34	36.00 ± 1.90	33.80 ± 2.41
Arterial pressure (mmHg)	83.20 ± 5.74	76.40 ± 1.52	78.20 ± 3.57	80.00 ± 3.68	76.80 ± 1.56	73.80 ± 1.51

Table 5

Summary of the statistical analysis outcomes for heart rate, cardiac output, ejection fraction, end-systolic volume and arterial pressure at 3 min and 15 min post US exposure. There were no significant interactions between sex and age for heart rate, cardiac output, systolic volume, ejection fraction, end-diastolic volume, end-systolic volume, respiratory rate and arterial pressure.

	Sex	Age	US	Interaction (Sex vs. Age vs. US)	Bonferroni
Heart Rate (3 min post US)	No	Yes**	Yes***	Yes***	Age & US P<0.001
Heart Rate (15 min post US)	No	No	Yes**	No	Sex & Age & US P<0.001
Cardiac Output (3 min post US)	No	Yes*	No	No	Sex & Age P<0.01
Cardiac Output (15min post US)	No	No	No	No	No
Ejection Fraction (3 min post US)	No	Yes**	Yes***	Yes**	US P< 0.001
Ejection Fraction (15 min post US)	Yes*	No	Yes***	No	US P< 0.001
End-Systolic Volume (3 min post US)	No	No	Yes***	Yes**	Sex & US P<0.001
End-Systolic Volume (15 min post US)	No	No	Yes***	No	Sex & US P<0.001
Arterial Pressure (3 min post US)	Yes**	No	No	No	No
Arterial Pressure (15 min post US)	No	No	No	No	No

Significant codes:

***
p < 0.001;

**
p < 0.01;

*
p < 0.05