# The Negative Chronotropic Effect in Rat Heart Stimulated by Ultrasonic Pulses

## Role of Sex and Age

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***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 approximately 1.0% at 2.0-MPa peak rarefactional pressure amplitude. 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 8 groups (n = 5 each): 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 US stimulation. Sex versus age versus 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. An hypothesis is that the US effect is weight (menopause) dependent, because the young (premenopausal) female rats weighed approximately 40 to 60% less than other groups of rats.

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

***Key Words***—age; biological effects; chronotropic effect; heart; sex; ultrasound

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**Abbreviations**
*PRF, pulse repetition frequency; US, ultrasound*

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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 the 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 with 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 (US) 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.[9–11] 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 with active control.[13] Previous studies in rat hearts demonstrated that transthoracic US can promote a negative chronotropic effect without impairing the cardiac pumping function.[14–17] 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 US has been shown to interfere in the cardiac activity of the turtle,[18] dog,[19] frog,[20] mouse,[21] pig,[22] guinea pig,[23] and rat.[14–17,24] 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 US/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 US to achieve leadless cardiac stimulation without adversely affecting cardiac tissue.

A more complete study of US-induced cardiac risk must take into account "premenopausal" and "post menopause" 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 US) 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, furthermore, interfere with possible therapeutic applications of controlled ultrasonic pulses in cardiology.

## Materials and Methods

### *Animals*

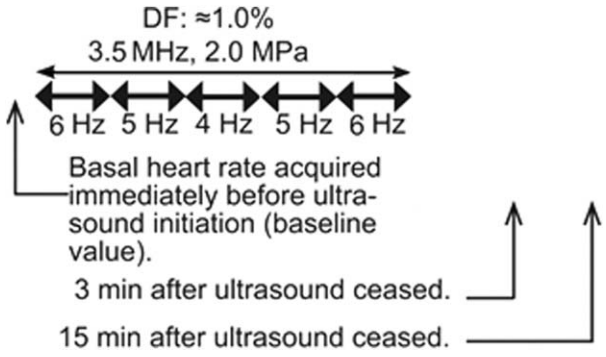
Experimental conditions were approved by the University of Illinois Institutional Animal Care and Use Committee (Protocol No. 10104). Forty F344 rats (Harlan, Indianapolis, IN) were divided into 8 groups ($n = 5$ each): Groups 1 and 2: 3-month-old 150 to 200 g (1) US young female and (2) control young female; Groups 3 and 4: 3-month-old 300 to 350 g (3) US young male and (4) control young male; Groups 5 and 6: 24-month-old 250 to 300 g (5) US old female and (6) control old female; and Groups 7 and 8: 24-month-old 400 to 500 g (7) US old male and (8) control old male. Animals received 5% isoflurane for inhalation anesthesia induction, and then 1.5 to 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 US application simultaneously. It is unlikely that this position would affect the heart rate or arterial pressure. Dorsal recumbency can compromise ventilation and $O_2$ transport, but the respiratory rate did not change significantly before and after the study.

The animals' limbs were secured to the electrocardiogram pads (4 leads) on the animal platform, a capability that is integral with the Vevo2100 high-frequency US imaging system (VisualSonics, Toronto, ON, Canada), 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 (eg, 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). In addition, 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 electrocardiogram were recorded continuously from before the initiation of the US application until approximately 15 min after the US exposure ceased (Figure 1). Then, at approximately 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.

**Figure 1.** Timeline for the 3.5-MHz transthoracic high-amplitude US stimulation. Baseline data were acquired before the initial US exposure. Postexposure data were acquired at 3 min and 15 min after US 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 is approximate, because the pulse duration was 2 ms.




### *Ultrasound*

An unfocused 19-mm-diameter aperture 3.5-MHz ultrasonic transducer was used (Valpey Fisher, Valpey Corp, Hopkinton, MA). The transducer was calibrated in a tank of distilled, degassed 22°C water. The calibrated hydrophone was a polyvinylidene fluoride 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-μm translational accuracy).[12]

The transducer was driven by a function generator (33250A, Agilent Technologies Inc, Santa Clara, CA) and a radiofrequency power amplifier (A500, Electronic Navigation Industries, Rochester, NY; 0.3–35 MHz; 55 dB). The Vevo2100 US imaging system, operated by a registered diagnostic medical sonographer, was used to monitor the heart using B-mode and M-mode displays. The Vevo2100 workstation has a tool to calculate the ejection fraction and other cardiac parameters by ventricular trace. To minimize thermal effects,[15] the following US protocol was applied (Figure 1): US bursts of 2.0-MPa peak rarefactional pressure amplitude (equivalent to an in vitro spatial peak temporal peak intensity of ~270 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 ~300–350 beats per min or 5 Hz), decreasing and increasing by 1-Hz steps. The duty factor was approximately 1.0%, which indicates that for a PRF sequence of 6, 5, 4, 5, and 6 Hz (ie, 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 rats,[26] for which the attenuation at 3.5 MHz was approximately 1.2 dB/cm MHz and the thickness was approximately 3.6 mm. Thus, the in situ ultrasonic pressure is 0.84 × 2.0 MPa = 1.7 MPa (Figure 2), and the in situ ultrasonic spatial peak temporal peak intensity is 0.70 × 270 W/cm$^2$ = 190 W/cm$^2$. Using the previously published procedures[15] with these in situ exposure parameters, the maximum in situ temperature increase from a single 2-ms-duration pulse is 0.009°C.

With an approximate 1% pulse duration, the steady-state in situ temperature increase is estimated to be 0.89°C, assuming normal heat removal processes.

### *Statistical Analysis*

The data were organized in a standard 2 (sex) × 2 (age) × 2 (US) factorial design with the following factors: sex (male versus female), age (young versus old), and US (on versus off) (Figure 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 among sex, age, and US at 3 and 15 min after US exposure compared with baseline for the following 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 the mean and standard error of the mean. The significance level was set at $P = .05$.

**Figure 2.** Transducer pressure response at approximately 2 MPa.

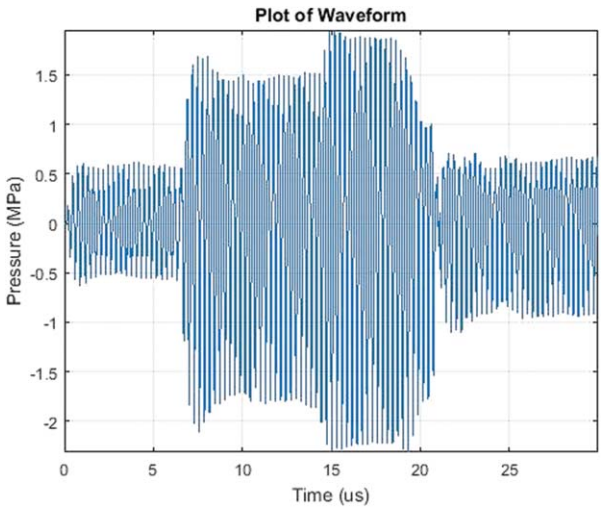



**Figure 3.** A 2 × 2 × 2 factorial design showing three factors: sex (male versus female), age (young versus old), and ultrasound (on versus off).

| | 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 |

## Results

The absolute values of physiological parameters at baseline and at 3 and 15 min after 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 after US exposure, the US effect (decrease of the heart rate) was significant ($P < .001$), the age effect was significant ($P < .01$), and the sex effect was not significant. The interactions among sex, age, and US were significant ($P < .001$). At 15 min after US exposure, the US effect (decrease of the heart rate) was significant ($P < .01$), the sex and age effects were not significant and there were no significant interactions (Figure 4A). The Bonferroni test showed a significant effect at 3 min after US exposure between age (young versus old) and US (on versus off). Moreover, at 15 min after US exposure the Bonferroni test showed a significant effect between sex (male versus female), age (young versus old), and US (on versus off).

Cardiac Output: At 3 min after US exposure, the US and sex effects were not significant, and the age effect was significant ($P = .03$). There were no significant interactions. At 15 min after US exposure, there were no significant effects or interactions (Figure 4B). The Bonferroni test showed a significant effect at 3 min after US exposure between sex (male versus female) and age (young versus old).

Ejection Fraction: At 3 min after US exposure, the US effect was significant ($P < .001$), the age effect was significant ($P < .01$), and the sex effect was not

**Table 1.** Absolute Values of Physiological Parameters at Baseline and at 3 and 15 Min After US Exposures for US Young Male and Control Young Male Groups

| | Control Young Male (N = 5) | | | US Young Male (N = 5) | | |
|---|---|---|---|---|---|---|
| **Parameter** | **Baseline** | **3 Min After US** | **15 Min After US** | **Baseline** | **3 Min After US** | **15 Min After 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 |

Note: See Figure 1. All values are expressed as mean and standard error of the mean. 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.** Absolute Values of Physiological Parameters at Baseline and at 3 and 15 Min After US Exposures for US Young Female and Control Young Female Groups

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

Note: See Figure 1. All values are expressed as mean and standard error of the mean. 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 3.** Absolute Values of Physiological Parameters at Baseline and at 3 and 15 Min After US Exposures for US Old Male and Control Old Male Groups

| | Control Old Male (N = 5) | | | US Old Male (N = 5) | | |
|---|---|---|---|---|---|---|
| **Parameter** | **Baseline** | **3 Min Post US** | **15 Min After US** | **Baseline** | **3 Min After US** | **15 Min After 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 |

Note: See Figure 1. All values are expressed as mean and standard error of the mean. 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.

significant. There were significant interactions among sex, age, and US ($P < .001$). At 15 min after US exposure, there were significant US ($P < .001$) and sex ($P = .03$) effects, but no significant interactions (Figure 4C). The Bonferroni test showed a significant effect at 3 and 15 min after US exposures between US (on versus off).

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

End-systolic volume: At 3 min after US exposure, the US effect was significant ($P < .001$). The interactions among sex and age and US were significant ($P < .01$). At 15 min after US exposure, there was a significant US effect ($P < .001$), and no significant sex or age effects or interactions (Figure 4E). The Bonferroni test showed a significant effect at both 3 and 15 min after US exposure between sex (male versus female) and US (on versus off).

Arterial Pressure: At 3 min post US exposure, there was a significant sex effect ($P = .01$). There were no significant age or US effects or interactions (Figure 4F). At 15 min after US exposure, there were no significant effects or interactions. The Bonferroni test showed no differences among age, sex, and US.

**Table 4.** Absolute Values of Physiological Parameters at Baseline and at 3 and 15 Min After US Exposures for US Old Female and Control Old Female Groups

| | Control Old Female (N = 5) | | | US Old Female (N = 5) | | |
|---|---|---|---|---|---|---|
| Parameter | Baseline | 3 Min After US | 15 Min After US | Baseline | 3 Min After US | 15 Min After 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 |

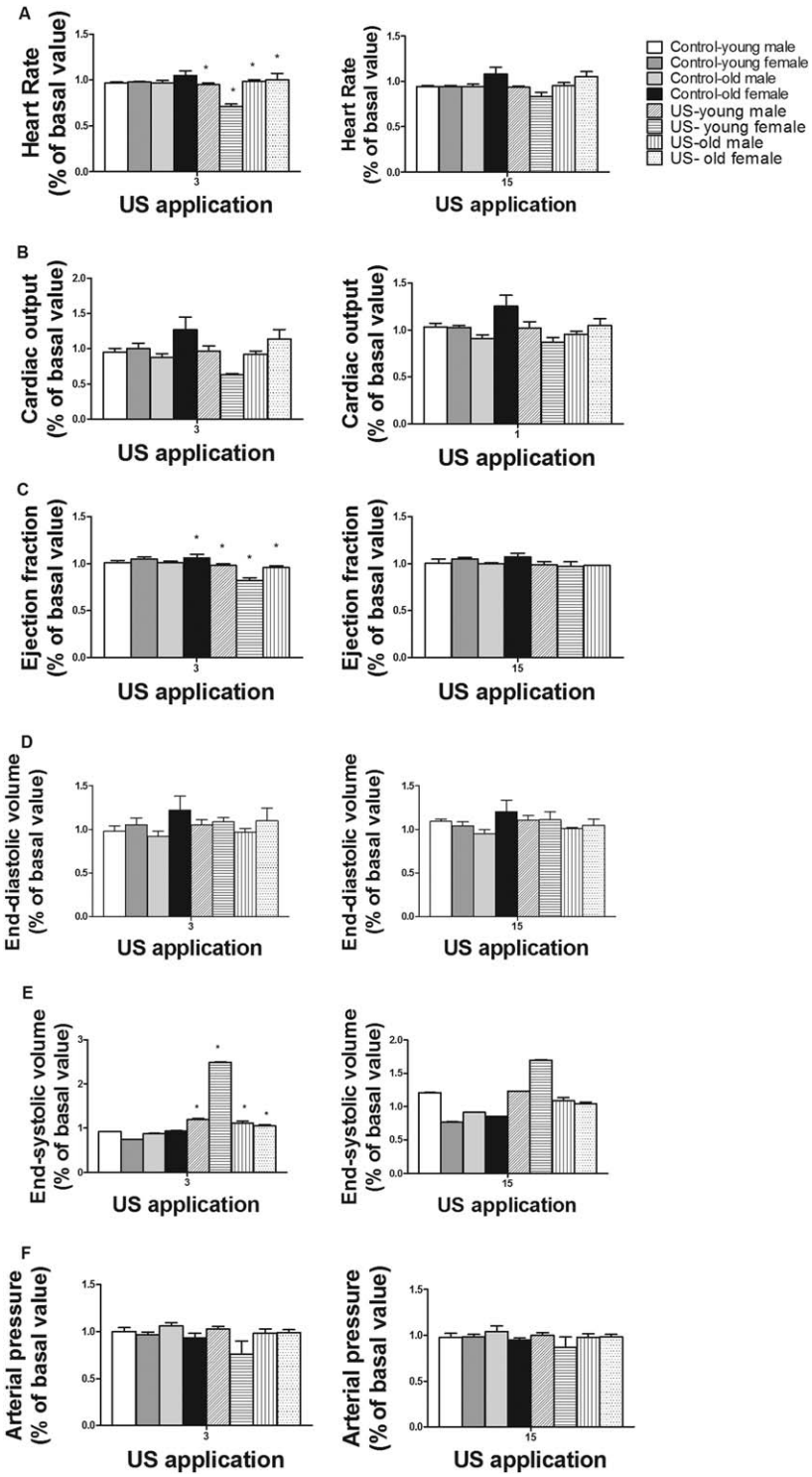
Note: See Figure 1. All values are expressed as mean and standard error of the mean. There were no significant interactions between sex and age for heart rate, cardiac output, systolic volume, ejection fraction, end-diastolic volume, end-systolic volume, respiratory rate, and arterial pressure.

**Table 5.** Summary of the Statistical Analysis Outcomes for Heart Rate, Cardiac Output, Ejection Fraction, End-Systolic Volume, and Arterial Pressure at 3 and 15 Min After US Exposure

| | Sex | Age | US | Interaction (Sex Versus Age Versus US) | Bonferroni |
|---|---|---|---|---|---|
| Heart Rate (3 min after US) | No | Yes[a] | Yes[b] | Yes[b] | Age and US $P < .001$ |
| Heart Rate (15 min after US) | No | No | Yes[a] | No | Sex and Age and US $P < .001$ |
| Cardiac Output (3 min after US) | No | Yes[c] | No | No | Sex andAge $P < .01$ |
| Cardiac Output (15 min after US) | No | No | No | No | No |
| Ejection Fraction (3 min after US) | No | Yes[a] | Yes[b] | Yes[a] | US $P < .001$ |
| Ejection Fraction (15 min after US) | Yes[c] | No | Yes[b] | No | US $P < .001$ |
| End-Systolic Volume (3 min after US) | No | No | Yes[b] | Yes[a] | Sex and US $P < .001$ |
| End-Systolic Volume (15 min after US) | No | No | Yes[b] | No | Sex and US $P < .001$ |
| Arterial Pressure (3 min after US) | Yes[a] | No | No | No | No |
| Arterial Pressure (15 min after US) | No | No | No | No | No |

Note: 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.
[a] $P < .01$.
[b] $P < .001$.
[c] $P < .05$.

**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 eight groups.



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

## Discussion

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

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

In addition, Table 5 indicates for heart rate, ejection fraction, and end-systolic volume that there was a significant interaction among sex versus age versus US. The negative chronotropic effect depends on sex, age, and US for heart rate, ejection fraction, and end-systolic volume. However, the Bonferroni test showed a significant effect at 3 min after US exposure between age (young versus old) and US (on versus off), and at 15 min after US exposure, the Bonferroni test showed a significant effect among sex (male versus female), age (young versus old), and US (on versus 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 after US exposures between US (on versus off) and interaction among sex, age and US, suggesting that the effect was caused by US. The Bonferroni test showed a significant effect at both 3 and 15 min after US exposure between sex (male versus female) and US (on versus 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, such as studies of 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 in the heart rate after US application, and some limitations might arise from this investigation.

In this study, a decrease in the heart rate (∼30%) was observed in the young female rats at 3 min after US exposure (Figure 4A). The US exposure parameters caused a cardiac output depression of approximately 37% and an ejection fraction decrease of approximately 18% at 3 min after US exposure. The end-systolic volume increased approximately 150% at 3 min after 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 appears 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 approximately 40% less, and compared with old males weighed approximately 60% less. Moreover, the size of the heart in the young females was smaller than the other rats (typical ranges are 1.5–2.5 cm in external length and 1.3–1.5 cm in external width).

Because the same US transducer was used (1.9-cm-diameter beam width remained unchanged because the heart was within the first 2 cm [10%] of the Fresnel zone; Fresnel zone was ∼20 cm for the 1.9-cm-diameter 3.5-MHz transducer), it is possible that the US field interacts 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 that the hormones respond differently between the sexes. Differences in animal models support the idea that sex hormones and their

steroid hormone receptors regulate this cardiovascular response.[25] A few studies that focused on cardiac hypertrophy showed a significant increase in cardiac mass in female rats[27] and female mice,[28–30] better contractile function in male rats,[31] 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 "postmenopausal" rats. It appears that the hormone contributions are critical in the observed outcomes.

In a previous study,[17] the intrathoracic (intercostal space) and rectal temperatures were monitored and no significant changes were observed after US exposure. Because the intrathoracic temperature measure is invasive and previous results did not show any changes, in this study only the rectal temperature was monitored. Histological examination of the heart and lungs of all animals exposed to US did not show any gross abnormalities following the US 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 US application may have occurred from parasympathetic stimulation. In this study, the US-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 in 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 shown in a previous study[16] was that the direct US 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 approximately 23% was observed for young female rats at 3 min after US exposure. With the decrease in 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 among sex, age, and US. 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 emphasize the need of additional studies to elucidate the physiological mechanisms involved in the production of these effects.

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