

Evaluation of infrared thermography, arterial Doppler ultrasound, and Doppler echocardiography in healthy adult dogs exposed to a single session of Whole-body vibration at different frequencies

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ABSTRACT

This study aimed to evaluate the infrared thermography, arterial Doppler ultrasound, and Doppler echocardiography in healthy adult dogs exposed to a single Whole-body vibration (WBV) at different frequencies. Sixteen (16) healthy crossbreed dogs males of ages 1 to 5 years, weighing 16.3 to 24.5 kg were enrolled in the study. The dogs were exposed to a single WBV session at frequencies of 30 Hz (5 min), 40 Hz (5 min), and 50 Hz (5 min) with intervals between each frequency exposure of 10 min. The cutaneous temperature, arterial Doppler ultrasound, and Doppler echocardiography were evaluated 10 min before the WBV session, between each frequency, and 1 min after the last frequency. The cutaneous temperature of the regions of the superficial gluteal muscle and biceps femoris muscle of both hind limbs was obtained with an infrared camera. Resistive indexes of carotid and femoral arteries were determined using Doppler ultrasound, and Doppler echocardiography was used to assess the end-systolic volume and end-diastolic volume, heart rate, aortic blood flow velocity, and pulmonary artery flow velocity. Clinical parameters, complete blood count, and serum biochemical (alanine aminotransferase, creatinine, and creatine phosphokinase) were evaluated 10 min before and 60 min after the end of the WBV session. Statistically significant differences were not found in any of the variables among the time points. In conclusion, the protocol of increasing vibration frequencies (30, 40, and 50 Hz) at short-period WBV can be considered appropriate since no change occurred in the parameters evaluated.

Introduction

Vibrating platforms have been used frequently in humans to promote whole-body vibration (WBV), aiming for aspects of health, physiotherapy, sports, and exercises (3, 4, 22). Several studies have been performed to understand the acute and chronic responses to WBV on cardiovascular indices (3, 4, 12). Although, it is necessary

to elucidate the effects of WBV in animals, particularly in dogs, since this type of exercise using vibrating platforms is already used in small animals (6, 10, 16-19, 23). Differences can occur among the different vibrating platforms (12, 13, 15), however, the vibrating frequency, amplitude, acceleration, and duration are variables that determine the intensity of the mechanical stimulus, being

the body position and posture also a critical elements, and is an absence of consensus on optimal parameters for humans (4, 5, 13). The authors have previously evaluated WBV in dogs considering its applicability potential in several disorders (6, 10, 16-19, 23), and to define safe protocols based on responses to vibration stimuli, the authors used the same device and kept the dog in a standing position over the vibrating platform.

Thus, this study aimed to evaluate the infrared thermography, arterial Doppler ultrasound, and Doppler echocardiography in healthy adult dogs exposed to a single WBV at different frequencies. The hypothesis was that the effects of increasing vibration frequencies would be reflected by an increase in echocardiographic and thermographic parameters and a decrease in the resistivity index. The clinical relevance of WBV programs is associated with physical condition improvement without joint damage, and muscle hypertrophy as report in human patients. Dogs diagnosed with muscle atrophy regrading to hip dysplasia can benefit from this modality as an additional form of conservative treatment for muscle atrophy and possible improvement of quality of life in dogs.

Material and Methods

Animal selection: The methodology adopted in this study was approved by the Institutional Ethics Committee on Animal Use of the School of Veterinary Medicine and Animal Science (FMVZ), São Paulo State University (Unesp), Botucatu, São Paulo, Brazil (n°. 0120/2018-CEUA). A written consent form was obtained from the dog's owner before starting the study. Sixteen (16) healthy crossbreed dogs males of ages from 1 to 5 years (mean \pm SD, 3.5 ± 2.5 years), and weighing 16.3 to 24.5 kg (mean \pm SD, 20.4 ± 4.1 kg) were enrolled in the study. The dogs were considered healthy based on their clinical history, physical examination, complete blood count (CBC), and serum biochemical [alanine aminotransferase (ALT), creatinine, creatine phosphokinase (CK)].

The inclusion criteria were dogs that had not been submitted to any surgical procedure for at least six months and had not taking medications for three months. Exclusion criteria included concerns raised by findings in the history and changes detected in the physical examination and/or laboratory tests.

Whole-body vibration protocol: No training was necessary for the dogs to get used to the vibrating platform. Before the WBV session, 6-hour fasting and 1-hour water restriction were stipulated. In addition, 30 min of rest was determined to acclimatize in the room environment with a controlled temperature (22° C) and humidity between 40 and 45%. The room had artificial light by LED lamps and blackout curtains to prevent

sunlight entrance. The WBV session was performed at 02:00 p.m. by the same operator.

The vibrating platform (92 cm length, 62 cm width, and 16 cm height) delivered a vortex wave circulation (TheraPlate Revolution, Texas, USA) that was used to promote the WBV. During the sessions, dogs remained on the top of the vibrating platform in a standing position without any sedation, and a leash was used to prevent them from moving or sitting (Figure 1). The dogs were exposed to single WBV at frequencies of 30 Hz [peak displacement (D_{peak}) = 3.10 mm; peak acceleration (A_{peak}) = 11.16 m s^{-2}] for 5 min, 40 Hz for 5 min (D_{peak} = 3.37 mm; A_{peak} = 21.59 m s^{-2} peak acceleration), and 50 Hz for 5 min (D_{peak} = 3.98 mm; A_{peak} = 39.75 m s^{-2}). The mean interval between each frequency exposure was 10 min. The frequency was determined by the manufacturer and checked by a digital oscilloscope (Mustool MDS120M, Mustool, California, USA), and for peak acceleration acquisition was used a 3-axis digital accelerometer sensor (STMicroelectronics, São Paulo, Brazil) was placed at the center of the vibrating platform. The peak displacement was calculated by using: $D_{peak} = A_{peak}/(2\pi f)^2$, f – frequency (4, 13).



Figure 1. Dog standing on the top of the vibrating platform which delivered a vortex wave circulation, and with a leash to prevent from moving or sitting.

Infrared thermography: The cutaneous temperature of the regions of the superficial gluteal muscle and biceps femoris muscle of both hind limbs was obtained with an infrared camera (FLIR Model E4, FLIR Systems, Boston, USA) with image resolution 256×256 pixels and thermal sensitivity of 0.07° C (Figure 2). The camera was positioned at 1 meter from the assessed regions with an angle of 90° while the dogs standing on the top of the vibration

platform. The room where the infrared thermography exams were performed and the acclimatization time in the room environment, temperature, and humidity were the same as the WBV sessions. During the exams, the room was maintained with the light on (led lamps) and blackout curtains, and only three persons were allowed in the room, to prevent variations in the room environment.

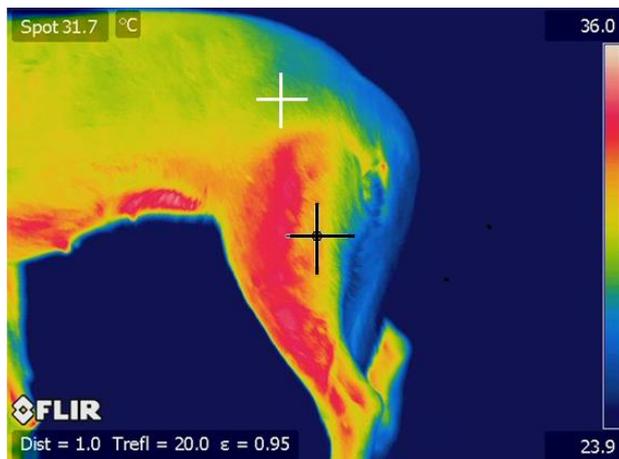


Figure 2. Cutaneous temperature of the regions of the superficial gluteal muscle (white spot) and biceps femoris muscle (black spot) of left hind limbs obtained with an infrared camera (image resolution = 256×256 pixels, thermal sensitivity = 0.07°C , distance from the assessed regions = 1 meter, angle = 90°).

The images were analyzed as spots in thermography software (Flir Systems v.1.2 software, FLIR Systems; Boston, USA). The same investigator carried out all thermographic examinations. The infrared thermography was performed at the following time points: 10 min before the WBV session, 2 min after starting, and 1 min after finishing with a frequency of 30 Hz; 2 min after starting and 1 min after finishing with a frequency of 40 Hz; 2 min after starting and 1 min after finishing with a frequency of 50 Hz.

Arterial Doppler ultrasound: Doppler ultrasonography of the common carotid and femoral arteries, on both the right and left sides, was performed using an ultrasound machine (MyLabTMFive, Esaote Healthcare; São Paulo, Brazil) with a 13 MHz multi-frequency linear transducer. The Resistivity Index (RI) of the common carotid artery was assessed in the mid-cervical region with the dog placed in lateral recumbency. Spectral Doppler sampling was performed with angles between 52° and 60° in the longitudinal plane. The RI of the femoral artery was evaluated with the dog on the top of the vibrating platform (turned off). After the femoral artery was identified in the femoral triangle area, the transducer was placed in the transverse plane. In both common carotid and femoral

arteries, ultrasound gel was used at the time of scanning. The RI was measured when three similar consecutive waveforms were obtained using triplex Doppler sonography. The ultrasound machine software calculated the RI automatically.

Doppler echocardiography: Doppler echocardiography assesses the end-systolic volume, end-diastolic volume, heart rate, aortic blood flow velocity, and pulmonary artery flow velocity. An ultrasound device (MyLabTMFive, Esaote Healthcare, São Paulo, Brazil) mode Triplex Doppler with an 8 MHz convex transducer was used.

The first evaluation was done between the fourth and fifth right intercostal spaces with the dog positioned in left lateral recumbency. Then, the evaluation was performed between the sixth and seventh left intercostal spaces with the dog positioned in the right lateral recumbency. Before ultrasound scanning, the acoustic gel was applied. The Arterial Doppler ultrasound and Doppler echocardiography parameters were measured 10 min before the WBV session and 1 min after finishing the WBV session in the frequencies of 30 Hz, 40 Hz, and 50 Hz. All sonographic examinations were performed by a single experienced operator with the dogs on top of the vibrating platform and in the same room of WBC sessions.

Clinical parameters: Clinical parameters included in the study were respiratory rate (RR), heart rate (HR), and body temperature (BT). All these parameters were measured with the dogs on top of the vibrating platform and assessed at 10-min before the WBV session, 1-min, 10 min, 30 min, and 60 min after the WBV session.

The RR was measured by counting the number of times the chest rose and falls, which combination counts were considered one breathes. The breaths were counted for 15 seconds and multiplied by four to get the respiratory rate in breaths per minute. The HR was measured using a veterinarian pulse oximeter (Prevtech[®], São Paulo, Brazil) which connected to the left dog's ear. For the BT parameters, a digital thermometer (G-Tech[®], Accumed Laboratory, São Paulo, Brazil) was inserted and placed in dog's rectum mucosa.

Blood analysis: Complete blood count (CBC) and serum biochemical (ALT, creatinine, CK) parameters were evaluated 10 min before and 60 min after the end of the WBV session. All blood samples were collected using a 21-gauge needle on a 10-mL syringe with the dog standing on the vibrating platform (turned off). Each blood sample (4 mL) for CBC was placed in a 5-mL plastic tube containing lithium heparin, and a blood sample (3 mL) for serum biochemical analysis was placed in a 5-mL plastic serum separator tube and centrifuged. Red blood cell

(RBC) values were determined by using an electronic cell counter (Ebram 18 hemascreeen, Ebram; São Paulo, Brazil), and the white blood cell (WBC) values were verified by using a Wright-stained blood smear and examined under an optical microscope (X100). Alanine aminotransferase and CK values were determined by using a commercial kit (Labtest; São Paulo, Brazil). Creatinine values were performed by applying a colorimetric method (Spectronic 21, Merck KGaA; Darmstadt, Germany).

Statistical analysis: Data normality was examined using the Shapiro-Wilk test. Friedman test was used to compare the values of infrared surface thermography, arterial Doppler ultrasonography, and echocardiography among the time points. Wilcoxon test compared clinical parameters, CBC values, and serum biochemical parameters between time points. Differences were considered significant at $P < 0.05$.

Results

The dogs maintained the standing position during the WBV session. However, they tried to sit while the vibration frequency was changed from 30 to 50 Hz. Signs of gastrointestinal conditions were not detected during and after the WBV session. No statistical differences were identified in the cutaneous temperature of regions of the superficial gluteal muscle and biceps femoris muscle of both hind limbs among time points (Table 1) ($P > 0.05$). On both the right and left sides, the RI values of the common carotid and femoral arteries did not show statistical variations among the time points (Table 2) ($P > 0.05$).

The values of end-systolic volume, end-diastolic volume, heart rate, aortic blood flow velocity, and pulmonary artery flow velocity did not show statistical differences among the time points (Table 3) ($P > 0.05$). No significant differences were found for clinical parameters (RR, HR, and BT) (Table 4), CBC values, and ALT, creatinine, and CK levels (Table 5) ($P > 0.05$).

Table 1. Cutaneous temperature ($^{\circ}$ C) measured by infrared thermography (CT – IR) of regions of the superficial gluteal and biceps femoris muscles at different time-points (TP).

CT – IR ($^{\circ}$ C)	TP0 (Mean \pm SD)	TP1 (Mean \pm SD)	TP2 (Mean \pm SD)	TP3 (Mean \pm SD)	TP4 (Mean \pm SD)	TP5 (Mean \pm SD)	TP6 (Mean \pm SD)	P value
SGM _{right}	32.37 \pm 0.92	33.68 \pm 1.31	33.50 \pm 1.33	33.80 \pm 1.59	33.69 \pm 2.14	33.69 \pm 2.14	33.36 \pm 1.81	0.2189
SGM _{left}	32.32 \pm 1.10	33.78 \pm 1.30	33.38 \pm 1.22	33.83 \pm 1.50	33.90 \pm 1.98	33.90 \pm 1.98	33.30 \pm 1.91	0.1372
BFM _{right}	36.01 \pm 0.50	36.17 \pm 0.40	36.22 \pm 0.50	36.23 \pm 0.38	36.48 \pm 0.77	36.48 \pm 0.77	35.67 \pm 0.92	0.1472
BFM _{left}	35.92 \pm 0.65	35.93 \pm 0.49	36.19 \pm 0.44	36.20 \pm 0.46	36.82 \pm 0.87	36.82 \pm 0.87	35.89 \pm 0.99	0.1243

SGM_{right} - Right superficial gluteal muscles, SGM_{left} – Left superficial gluteal muscles, BFM_{right} – Right biceps femoris muscles, BFM_{left} – Left superficial biceps femoris muscles. TP0 - 10 min before WBV session; TP1 - 2 min after starting WBV (30 Hz); TP2 - 1 min after finishing WBV (30 Hz); TP3 - 2 min after starting WBV (40 Hz); TP4 - 1 min after finishing WBV (40 Hz); TP5 - 2 min after starting WBV (50 Hz); TP6 - 1 min after finishing WBV (50 Hz).

Table 2. Arterial Doppler ultrasound values at different time-points (TP).

RI	TP0 (Mean \pm SD)	TP1 (Mean \pm SD)	TP2 (Mean \pm SD)	TP3 (Mean \pm SD)	P value
CA _{right}	0.87 \pm 0.04	0.86 \pm 0.05	0.85 \pm 0.06	0.85 \pm 0.07	0.2108
CA _{left}	0.87 \pm 0.04	0.87 \pm 0.05	0.86 \pm 0.07	0.86 \pm 0.05	0.6489
FA _{right}	0.94 \pm 0.02	0.92 \pm 0.02	0.92 \pm 0.05	0.92 \pm 0.03	0.1865
FA _{left}	0.94 \pm 0.01	0.90 \pm 0.07	0.93 \pm 0.03	0.90 \pm 0.02	0.1928

RI - Resistive index, CA_{right} – Right common carotid artery, CA_{left} – Left common carotid artery, FA_{right} – Right femoral artery, FA_{left} – Left femoral artery. TP0 - 10 min before WBV session; TP1 - 1 min after finishing WBV (30 Hz); TP2 - 1 min after finishing WBV (40 Hz); TP3 - 1 min after finishing WBV (50 Hz). Reference values - RI of common carotid artery: 0.83 – 0.91; RI of common femoral artery: 0.92 – 0.96.

Table 3. Doppler echocardiography values at different time-points (TP).

Parameters	TP0 (Mean \pm SD)	TP1 (Mean \pm SD)	TP2 (Mean \pm SD)	TP4 (Mean \pm SD)	P value
ESV (mL)	17.22 \pm 16.98	25.62 \pm 19.39	45.28 \pm 56.88	45.94 \pm 59.62	0.2590
EDV (mL)	53.00 \pm 28.85	68.05 \pm 36.48	81.96 \pm 57.29	79.69 \pm 61.72	0.3420
HR (bpm)	117.44 \pm 18.56	115.22 \pm 15.67	127.22 \pm 23.78	113.44 \pm 14.49	0.0858
AoV (cm/sec)	146.33 \pm 36.92	147.00 \pm 44.37	146.44 \pm 31.80	148.22 \pm 33.57	0.9816
PulmV (cm/sec)	124.22 \pm 33.03	110.11 \pm 29.91	106.00 \pm 43.69	109.00 \pm 37.99	0.2185

ESV - end-systolic volume; EDV - end-diastolic volume; HR - heart rate; AoV - aortic blood flow velocity; PulmV - pulmonary artery flow velocity. TP0 - 10 min before WBV session; TP1 - 1 min after finishing WBV (30 Hz); TP2 - 1 min after finishing WBV (40 Hz); TP3 - 1 min after finishing WBV (50 Hz). reference values – ESV: 0.22 – 34.22 mL; EDV: 24.00 – 82.00 mL; HR: 60 – 160 bpm; AoV: 109.00 – 183.00 cm/sec; PulmV: 91 – 157.00 cm/sec.

Table 4. Clinical parameters at different time-points (TP).

Parameters	TP0 (Mean ± SD)	TP1 (Mean ± SD)	TP2 (Mean ± SD)	TP3 (Mean ± SD)	TP4 (Mean ± SD)	P value
RR (mpm)	19.7 ± 2.1	23.7 ± 1.4	24.8 ± 1.3	18.9 ± 1.8	17.6 ± 2.3	0.120
HR (bpm)	100.4 ± 21.2	110.9 ± 21.6	106.9 ± 19.1	91.9 ± 11.4	90.4 ± 20.1	0.222
BT (° C)	38.5 ± 0.4	38.8 ± 0.3	38.7 ± 0.6	38.4 ± 1.1	38.6 ± 1.12	0.066

RR – Respiratory rate; CK; HR – Heart rate; BT – Body temperature. TP0 - 10 min before WBV session, TP1 – 1- min after a WBV session, TP2 – 10 min after WBV session, TP3 - 30 min after WBV session, TP4 - 60 min after WBV session. Reference values – RR - [18 – 36 movements per minute (mpm)]; HR - [60 – 160 beats per minute (bpm)]; BT: 37.5 – 39.2° C.

Table 5. Complete blood count and serum biochemistry values at different time-points (TP).

Parameters	TP0 (Mean ± SD)	TP1 (Mean ± SD)	P value
Erythrocytes (x10 ⁶ /μ)	7.42 ± 0.36	7.16 ± 0.29	0.1225
Hemoglobin (g/dL)	16.98 ± 0.99	16.21 ± 0.86	0.1156
Hematocrit (%)	50.00 ± 2.74	48.44 ± 1.13	0.1206
Total protein (g/dL)	7.08 ± 0.60	7.12 ± 0.32	0.1071
Leukocytes (x10 ³ /μL)	10.27 ± 0.98	10.36 ± 0.24	0.9052
Neutrophils (x10 ³ /μL)	7.12 ± 0.15	7.53 ± 0.23	0.5519
Eosinophils (x10 ³ /μL)	0.92 ± 0.44	1.03 ± 0.13	0.6341
Lymphocytes (x10 ³ /μL)	1.66 ± 0.59	1.46 ± 0.80	0.4753
Monocytes (x10 ³ /μL)	0.56 ± 0.41	0.31 ± 0.12	0.3411
ALT (U/L)	43.78 ± 4.97	37.33 ± 6.33	0.2340
Creatinine (mg/dL)	1.13 ± 0.21	1.10 ± 0.13	0.3398
CK (U/L)	159.33 ± 111.46	188.778 ± 106.99	0.9050

ALT - Alanina aminotransferase; CK - Creatine phosphokinase. TP0 - 5 min before WBV session, TP1 - 60 min after a WBV session. Reference values - Erythrocytes: 5.60 – 8.70 x10⁶/μ; Hemoglobin: 14.0 – 20.0 g/dL; Hematocrit: 40.0 – 59.0%; Total protein: 5.40 – 7.10 g/dL; Leukocytes: 6.00 – 17.00 x10³/μL; Eosinophils: 0.00 – 1.90 x10³/μL; Lymphocytes: 0.53 – 4.80 x10³/μL; Monocytes: 0.10 – 1.80 x10³/μL; ALT: 10.00 – 88.00 U/L; Creatinine: 0.50 – 1.50 mg/dL; CK: 20.00 – 200.00 U/L.

Discussion and Conclusion

The present study evaluated infrared thermography, arterial Doppler ultrasound, and Doppler echocardiography in healthy adult dogs exposed to a single whole-body vibration (WBV) at different frequencies. Recently, vibrating platforms that produce mechanical vibrations and spread throughout the whole body have been researched for various purposes, including rehabilitation and exercise in small animals (1, 6, 10, 16-19, 23). The hypothesis was not confirmed in this study, as all parameters evaluated did not change despite the increasing vibration frequencies of WBV in a short time. The dogs showed good adaptation to the vibrating platform, as verified in other studies using the same equipment to assess short- and long-term responses (6, 17). However, changes in the dogs' behavior due to an increase in vibration frequency were also described in other studies. These changes were attributed to the pressure exerted on the paws (18) or possible paraesthesia (23). In a study involving healthy adult humans, a reduction in touch-pressure sensitivity of the feet was noted 10 minutes after WBV exposure (20).

No signs of gastrointestinal conditions were identified in the dogs in the present study. This finding is consistent with a study involving adult and elderly dogs exposed to 30 Hz and 50 Hz in a single WBV session (18). Considering that the suppression of gastric smooth muscle activity and contractions has been observed in humans exposed to short-term WBV (9), it is essential to evaluate the influence of food intake during WBV in dogs.

Although infrared thermography is considered a non-invasive technology used in sports and exercise science in humans, the analysis can be affected by the selection of regions of interest in anatomical areas (11). In the present study, infrared thermography of both the superficial gluteus muscle and biceps femoris muscle did not show changes in mean cutaneous temperature values during the evaluation time points. This indicates that the tested vibration frequencies in a single session were not sufficient to promote significant superficial vascular changes in these muscle regions. Notably, healthy Beagle dogs exposed to WBV sessions for five days, despite differences in vibration frequencies, also did not exhibit thermographic changes in the biceps femoris and vastus

lateralis muscles (17). In contrast, different results were observed in Beagle dogs subjected to high-speed physical exercise on a treadmill, which showed an increase in surface temperature, with the thigh being one of the most influenced sites (21). On the other hand, a decrease in lower limb temperature was found in humans during 15 minutes of WBV exposure at frequencies of 31, 35, 40, and 44 Hz, which was attributed to vasoconstriction (20). As reported by these authors, although WBV exercise has been compared to aerobic exercises, the responses to the same variables can be different.

In this study, no differences were detected in the Resistance Index (RI) of the femoral and common carotid arteries at frequencies of 30 Hz, 40 Hz, and 50 Hz ($P>0.05$). A study conducted in healthy dogs subjected to a single WBV session, using frequencies of 30 Hz, 50 Hz, and 30 Hz with exposures of five minutes each, also demonstrated no changes in renal RI (6). In contrast, healthy dogs exposed to the same frequencies and duration of WBV, as reported by Freire et al. (6), exhibited an increase in RI of the femoral artery from the second to the fifth day, suggesting reduced blood flow in the region of the quadriceps femoris muscles (17).

The absence of variations in end-systolic and end-diastolic volumes, heart rate, aortic blood flow velocity, and pulmonary artery flow velocity can be considered strong indicators that the vibration frequencies used in the present study were safe. Heart rate, systolic blood pressure, and ambulatory electrocardiography evaluated in neutered young and aged dogs were not modified at frequencies of 30 Hz (5 min), 50 Hz (5 min), and 30 Hz (5 min) during 15 minutes of WBV (19). Additionally, systolic blood pressure and mean heart rate did not show changes in healthy humans exposed to 30 minutes of vibration at 60 Hz (2). In contrast, healthy humans submitted to exercise until exhaustion on a vibration platform exhibited elevated heart rate and increased systolic blood pressure; however, these effects were considered mild and returned to normal within 15 minutes of recovery (14).

No significant variations ($P>0.05$) were identified regarding the values of clinical parameters, including RR, HR, and BT, which is similar to a study conducted in healthy younger and older adult male non-athletic dogs (19) and human patients (2). Nevertheless, it was observed that WBV encouraged increases in RR, HR, and BT after the WBV session. On the other hand, a study that used long-term WBV demonstrated an increase in HR of up to 30% after the WBV session (14). This suggests that short-term WBV does not induce significant changes in cardiac function. However, it would be important to evaluate cardiac troponin I (cTnI) to identify possible deleterious effects on the myocardium. In the present study, cTnI was not evaluated due to the absence of significant variations

in healthy dogs (both younger and adult) who used the same vibrating platform and short-term WBV protocols as those in the present study (19).

The CBC values did not show differences over time ($P>0.05$). However, a study with healthy Beagles subjected to WBV for five days showed a decrease in erythrocyte and hemoglobin values within the reference intervals. This decrease was attributed to hemolysis due to the action of mechanical vibration. In the same study, an increase in leukocyte, neutrophil, and eosinophil values was identified in these animals, which was associated with the release of epinephrine and norepinephrine as a result of acute stress (16).

Regarding serum biochemical values (ALT, creatinine, and CK), differences over time were also not detected ($P>0.05$). However, a study in humans during an ultramarathon race showed an increase in CK values, which was used as a marker of muscle injury (7, 8, 24). As previously discussed, the responses to WBV as exercise and traditional aerobic exercises may not be the same (20). This data is important since the use of WBV in dogs may help prevent severe muscle damage.

Lactate dehydrogenase and aspartate aminotransferase are important parameters when evaluating muscle functions in animals and human patients. However, the present study did not evaluate these parameters due to budget limitations. Therefore, it is important to include these parameters in future studies.

In conclusion, the protocol of increasing vibration frequencies (30, 40, and 50 Hz) in short-duration WBV, using a vibrating platform that delivered a vortex wave circulation, can be considered appropriate since no changes occurred in the evaluated parameters.

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Ethical Statement

This study was approved by the Institutional Ethics Committee on Animal Use of the School of Veterinary Medicine and Animal Science (FMVZ), São Paulo State University (Unesp), Botucatu, Brazil (n°. 0120/2018-CEUA).

Conflict of Interest

The authors declared that there is no conflict of interest.

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