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Short-term effects of Whole-Body Vibration on clinical, cardiac, and ambulatory electrocardiographic (Holter) parameters of healthy younger and older adult male non-athletic crossbreed dogs

[*Efeitos agudos da Vibração de Corpo Inteiro nos parêmetros clínico, cardíaco e eletrocardiografia ambulatorial (Holter) em cães jovens e idosos sem raça definida, hígidos e não atletas*]

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ABSTRACT

This study investigated the effects of WBV in clinical, cardiac, and ambulatory electrocardiographic (Holter) parameters of healthy young and aged male non-athletic dogs. Fourteen dogs were divided into two groups of seven animals: Group I (GI) – young dogs (12.0 and 84.0 months old); Group II (GII) – aged dogs (above 84.0 months old). Dogs were submitted to a single session of WBV (frequencies of 30 and 50 Hz), for 15-min. Variations were identified in the thickness of the interventricular septum and thickness of the left ventricle-free wall values: GI < GII at 5-min before the session. The diameter of the left atrium values showed a difference: GI < GII at 5-min before and 1-min after the session; and a decrease in GII between 5-min before and 1 min after the WBV. Several ambulatory electrocardiography (Holter) parameters demonstrated significant differences between both groups and time-points. A single session of WBV at frequencies of 30 and 50 Hz during 15-min by using a vibrating platform that delivered a vortex wave circulation did not induce significant changes in clinical, cardiac, and ambulatory electrocardiographic (Holter) parameters in healthy young and aged dogs.

Keywords: blood pressure, heart rate, non-pharmacological therapies, vibrating platform

RESUMO

O estudo teve como objetivo avaliar os efeitos agudos da vibração de corpo inteiro (VCI) nos parâmetros clínicos, cardíacos e eletrocardiográficos ambulatoriais (Holter) de cães jovens e adultos hígidos sem raça definida e não atletas. Quatorze cães foram divididos em dois grupos de sete cães, de acordo com a idade: Grupo I (GI) – entre 12,0 e 84,0 meses; Grupo II (GII) - acima de 84,0 meses. Os cães foram submetidos à única sessão de VCI com frequências de 30 e 50 Hz, durante 15 minutos. Foram identificadas variações significativas na espessura do septo interventricular e da espessura da parede do ventrículo esquerdo: GI <GII, cinco minutos antes da VCI. Os valores do diâmetro do átrio esquerdo demonstraram diferença: GI <GII, entre cinco minutos antes e um minuto após à sessão; e diminuição no GII entre cinco minutos antes e um minuto após à VCI. Vários parâmetros da eletrocardiografia ambulatorial (Holter) demonstraram diferenças signiicativas entre os dois grupos e momentos avaliados. Sessão única de VCI nas frequências de 30 e 50 Hz durante 15 minutos usando a plataforma vibratória de circulação vórtex não induziu alterações significativas nos parâmetros clínicos, cardíacos e eletrocardiográficos ambulatorial (Holter) em cães jovens e idosos hígidos.

Palavras-chave: pressão arterial, frequência cardíaca, plataforma vibratória, terapias não farmacológicas

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INTRODUCTION

Whole-body vibration (WBV) is a result of mechanical vibrating stimulus generated by vibrating platforms that propagate throughout the body (Rauch *et al.*, 2010; Rittweger, 2010; Sitjà-Rabert *et al*., 2012; Wuestefeld *et al.*, 2020), and used for physical rehabilitation (Cochrane, 2011). Regarding the beneficial effects on the cardiovascular system in human patients, Maloney-Hinds *et al.* (2008) identified an increase in skin blood flow using WBV protocol of three bouts of 1-min vibrating frequency of 30 Hz or 50 Hz, which maintained high following 10-min after de vibrating stimuli. Other positive effects of WBV were observed on muscle perfusion of popliteal, gastrocnemius, and vastus lateralis muscle by using a frequency of 26 Hz (peak displacement = 3 mm) and were suggestive of arterial vasodilation in the vibrated musculature (Kerschan-Schindl *et al*., 2001). A study conducted by Cardinale *et al*. (2007) concluded that WBV exercise induces muscle deoxygenation only at frequencies below 30 Hz.

WBV exercise improves the cardiovascular system without increasing the risk of cardiac injury (Wong *et al*., 2016; Moreira-Marconi *et al.*, 2020), and is indicated for aged subjects who are unable to comply with conventional training routines and patients who cannot perform traditional resistance training (Sitjà-Rabert *et al*., 2012; Park *et al*., 2015). Few studies evaluated the effects of WBV in dogs (Freire *et al.*, 2015; Santos *et al*., 2017, 2019; Nagai *et al*., 2020). A study by Freire *et al.* (2015), Nagai *et al.* (2020) on healthy adult dogs showed no alteration in the renal and common carotid resistive index, respectively, after a single session of WBV (30 and 50 Hz for 15-min) using a vibrating platform that delivered a vortex wave circulation. However, the femoral resistive index increased significantly in healthy beagle dogs submitted to the same WBV protocol for uninterrupted five days (Santos *et al*., 2019). Silva *et al.* (2020), Tannus *et al.* (2021) did not identify significant changes in haematobiochemical and hemogasometric, and cortisol values, respectively, in healthy dogs submitted to a single session of WBV (30 and 50 Hz frequencies for 15 min) by using a vibrating device with vortex movement. Additionally, Blazizza *et al.* (2020) conclude that 15 min sessions of WBV at frequencies varied between 30 and 50 Hz did not induce significant alteration in haematological and serum cortisol levels in healthy adult cats.

Since WBV can be used as a resistance exercise for cardiovascular improvement in dogs, and cardiac and ambulatory electrocardiographic (Holter) parameters can be used to evaluate indirectly the muscular system. This study evaluated the acute effects of WBV on clinical, cardiac, and ambulatory electrocardiographic (Holter) parameters of healthy young and aged neutered male non-athletic crossbreed dogs submitted to the vibrating platform that delivered a vortex wave circulation. So far, to the best of the authors' knowledge, no data exist regarding the WBV effect of these cardiac parameters in dogs. On the other hand, studies should be conducted to obtain better scientific evidence for establishing effective protocols for WBV application in dogs since in human patients there are a variety of protocols and vibrating platforms for different conditions (Rauch *et al.*, 2010). The hypothesis was that a single session of WBV (30 and 50 Hz, 15-min) will not induce significant variations on clinical and cardiovascular parameters in these dogs.

MATERIALS AND METHODS

The study was approved by the Animal Ethics Commission of the School of Veterinary Medicine and Animal Science, São Paulo State University, São Paulo, Brazil (Number 0133/2017), and conducted in the following GPS location: latitude: -22.888941850000002; longitude: -48.49840491716181.

Fourteen clinically healthy, non-athlete neutered crossbreed male dogs were used in the study. The dogs belonged to our laboratory and all of them had the same walking and human contact protocol. The non-athlete condition was determined by the time of walking (20-min on the leash each 24 h), and the sociable and docile condition was determined by human contact of 45-min daily. After the WBV session, they remained isolated in a dog kennel with 2.5 m^2 and free access to water and food without human contact until the end of the study. All animals received 50 to 300 gram per day of the same brand of dry food.

Inclusion criteria: Dogs with an absence of changes in clinical, orthopedic, neurological, and echocardiographic exams, and with physiological

parameters of hemogram and serum biochemistry (alanine aminotransferase, total serum protein, albumin, urea, creatinine, creatine kinase, and cardiac troponin I) were included in the study.

Exclusion criteria: Dogs submitted to any surgical procedure at least 12 months before the study or receiving any medication at least six months before the study, and with a body condition score below three points or above five points in the 9-point body condition scoring (Baldwin *et al*., 2010) were removed from the study.

Experimental procedures: Fourteen dogs were divided into two groups of seven dogs: Group 1 (G1) - young dogs (27.40 ± 5.90 months old) with body mass (BM) of 22.81 ± 3.40 kg; Group 2 (G2) aged dogs (96.00 ± 13.90 months old) with BM of 27.82 ± 8.80 kg.
WBV: Before the WBV session, all dogs fasted for six hours, and water was withheld for four hours. The dogs were submitted to physical rest and acclimatization on top of the vibrating platform (disconnected) for one hour before the WBV. The sessions were performed in a room with a constant 22° C and humidity between 40 and 45% measured by a digital hygrometer (Mettler-Toledo®, São Paulo, Brazil). In the room where the study was carried out, noise was limited and no more than three persons were allowed in the space at a time.

No training was necessary for the dogs to get used to the vibrating platform. The dogs were submitted to a single WBV session by using a vibrating platform (TheraPlate®, Texas, USA) (length = 92cm, width = 62cm, height = 16 cm), which delivered a vortex wave circulation. The frequency was set by the manufacturer and varied from 05 to 100 Hz and was checked using a digital oscilloscope (Mustool MDS120M®, California, USA). The peak acceleration (Apeak) was determined using a 3-axis digital accelerometer sensor (STMicroelectronics®, São Paulo, Brazil), and peak displacement (Dpeak) was calculated using the following formula: Dpeak = Apeak/(2.f)2, f – frequency (Rauch *et al*., 2010; Cochrane, 2011). The velocity (V) was determined by the formula: V = Dpeak.π.f (π = 3.14) (Rauch *et al*., 2010; Cochrane, 2011).

The WBV protocol started with a frequency of 30 Hz (Dpeak = 3.10 mm; Apeak =11.16 m/s^2; V = 0.29 m/s) for 5-min, followed by an increase to 50 Hz (Dpeak = 3.98 mm; Apeak = 39.75 m/s^2; V = 0.62 m/s) for 5-min and finishing with a frequency of 30 Hz (Dpeak =3.10 mm; Apeak =11.16 m/s^2; V = 0.29 m/s) for 5-min, without rest (Freire *et al.*, 2015; Santos *et al*., 2017; Santos *et al.*, 2019; Nagai *et al.*, 2020; Silva *et al.*, 2020). During the WBV session, the dogs remained standing on the center of the vibrating platform. Dogs were prevented from walking away through a leash which avoiding restricted airways (Santos *et al*., 2019; Nagai *et al*., 2020). The clinical, ambulatory electrocardiographic (Holter), and echocardiographic parameters were evaluated in the room where the study was carried out.

Clinical parameters and cardiac troponin I (cTnI): Clinical parameters included respiratory rate (RR), heart rate (HR), body temperature (BT), and systolic blood pressure (SBP). RR was measured by observation and counting the number of times the chest rises (inhales) and falls (exhales), and each rise/fall combination counts as one breathes. The breaths were counted for 30 seconds and multiplied by two 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 right dog's ear. An infrared thermometer (Fora FocusTemp®, Gallen, Switzerland) was used to assess the BT inside of the left ear. Systolic blood pressure was determined using a veterinary Doppler (Martec Med, Ribeirão Preto, Brazil) according to Acierno *et al*. (2018). All parameters were assessed at 5-min before WBV session (5PRE), 1-min (1POST), 10 min (10POST), 20 (20POST), 30 min (30POST), 60 min (60POST), 120-min (120POST), 360 min (360POST), 12 h (12hPOST), and 24 h after WBV session (24hPOST). The reference ranges used by our laboratory were RR [18 – 36 movements per minute (mpm)], HR [60 – 160 beats per minute (bpm)], BT (37.5 – 39.2º C), and SBP (80.50 – 130.30 mmHg).

Four milliliters of blood samples were obtained from a jugular vein using a 21-gauge needle on a 5 mL syringe and dogs standing on the vibrating platform at different time-points: 5PRE, 1POST, 30POST, 60POST, 120POST, 360POST, 12hPOST, and 24hPOST. Blood samples were placed in 5 mL plastic tubes containing lithium heparin and immediately analyzed. Cardiac troponin I value was determined by enzyme-linked immunosorbent assays (ELISA) method

using a commercial kit (BioMérieux®, Rio de Janeiro, Brazil) according to manufacturer instructions. The reference ranges established by our laboratory for healthy dogs were 0.11 to 0.16 ng/mL (Hamacher *et al.*, 2015). All exams were evaluated by the same resident of the institution's clinical analysis laboratory with dogs standing on top of the vibrating platform turned off to avoid any physiological alteration concerning stress.

Echocardiographic parameters: The echocardiographic examination was performed using an ultrasound device (GE®, São Paulo, Brazil) with Doppler function, and 2 – 8 MHz multifrequency sectorial transducer. The measurements were taken with animals in recumbency position during diastole through the right parasternal window in a transverse section using an M-mode.

The thickness of the interventricular septum (IVSd), the internal diameter of the left ventricle (LVIDd), and the thickness of the left ventricular wall (LVFWd) were variables measured. The thickness of the interventricular septum (IVSs), the internal diameter of the left ventricle (LVIDs), and the thickness of the left ventricular wall (LVFWs) were measured during systole. The diameter of the left atrium (LA) during systole, the diameter of the aorta (Ao) during diastole, left atrium/aorta ratio (LA/Ao), left ventricle-shortening fraction (LVSF), ejection fraction (EF), pulmonary flow velocity (PFV) and the pressure gradient between the right ventricle and pulmonary artery (pulmonary pressure gradient) (PPG) were also recorded. The following formula was used to calculate the LVSF: (LVIDd-LVIDs/LVIDd) x 100. Other echocardiographic parameters included the mitral flow of E-wave (Vel E_mitr), deceleration time of E-wave (TDEm), mitral flow of A-wave (VelA_mitr), isovolumetric relaxation time (TRIV), the ratio of the E-wave and A-wave (E_A), fast and slow ventricular filling time (E_sep_VE) and atrial contraction (A_sep_VE). The means were calculated based on three measurements, all taken by the same operator. These parameters were measured at the following time-points: 5PRE, 1POST, 12hPOST, and 24hPOST. These echocardiographic parameters were evaluated with the dogs standing on the top of the vibrating platform turned off and performed by the same person.

Ambulatory electrocardiographic (Holter): The ambulatory electrocardiographic (Holter) parameters evaluated were the minimum, average and maximum HR (HRmin, HRaver, and HRmax); the interval between two consecutive N-waves of the entire record (NNm); normal RR intervals every 5-min (SDNNIDX); normal RR intervals (NNs); RR wave intervals (SDNN); interval mean NN-wave obtained every 5-min (SDANN); differences between adjacent normal RR or NN intervals (rMSSD); the percentage of adjacent RR or NN intervals with a difference in duration greater than 50 milliseconds (pNN > 50).

These parameters were measured using an ambulatory Holter (Cardios®, São Paulo, Brazil) (dimensions: 82 x 60 x 14 mm) with digital recording of three channels and signal acquisition of 800 pps-12 bits, and with pacemaker detection circuit with an internal clock and infrared transmission. Two electrodes were attached between the 3rd and 4th right and left intercostal space and two others between the 4th and 5th right and left intercostal space. White and black electrodes were placed on the left side and the green and red electrodes on the right side. The electrode wires were connected to the Holter, and it was positioned in the region between the 3rd and 4th thoracic vertebrae and fixed with tape and wrapped with an elastic bandage. The measurements were stored on the device's SD card and analyzed by the same person. The measurements were performed using the dogs standing on top of the vibrating platforms without being connected and by the same person at six time-points: 5PRE, 1POST, 120POST, 360POST, 12hPOST, and 24hPOST. During the ambulatory electrocardiographic (Holter) assess the dogs were submitted to physical rest and remained isolated in a doghouse with 2.5 m^2 with free access to water and food, and no contact with people. The reference ranges were HRmin (30.0 −58.0 bpm); HRaver (81.0 – 105.0 bpm); HRmaxim (235.0 – 250.0 bpm); NNm (482.0 – 845.0 m/s); SDNNIDX (161.0 – 359.0 m/s); NNs (79.0 −130.0 x103 m/s); SDNN (137.4 - 322.3 m/s); SDANN (148.0 – 202.0 m/s); rMSSD (90.0 – 155.0 m/s); pNN > 50 (48.0 – 69.0).

Statistical analysis: Statistical analysis was performed using the R software, Version 3.4.4 (2018-03-15). The normality of the variables recorded at each time-point and group was assessed by the Shapiro-Wilks test. For all

analyzes, a significant difference was considered when the *p*-value was less than 0.05. The comparison between the time-points for each group was performed by adjusting the mixed model considering the time-point as a random effect and then the multiple comparisons between the time-points. Mann–Whitney test was used to compare each group.

RESULTS

Clinical parameters and cardiac troponin I (cTnI): Simply anecdotal evidence showed that aged dogs with body mass above 30 kg (92.9%) tried to sit and walk after 3-min at a frequency of 50 Hz. No animals tried to jump out of the vibrating platform and no diarrhea or vomiting was observed. Regarding the dog's behavior, 71% of the dogs in each group remained calm and 29% agitated, immediately after the WBV session. There were no significant variations in RR, HR, BT, SBP, and cTnI values in young and aged dogs at all time points (Tab. 1).

Echocardiographic parameters: A significant variation was observed in IVSd and LVFWd values: GI<GII ($p = 0.0113$) at 5PRE (Tab. 2). LA values showed a significant difference: GI<GII ($p < 0.01$) at 5PRE and 1POST; and a decrease ($p < 0.01$) in GII between 5PRE and 1POST (Tab. 3). Other echocardiographic parameters did not show differences (Tab. 3, 4).

Table 1. Mean and standard deviation (Mean ± SD) of RR, HR, BT, SBP and cTnI at 5PRE, 1POST, 10POST, 20POST, 30POST, 60POST, 120POST, 360POST, 12hPOST and 24hPOST, in young (GI, n = 7) and aged dogs (GII, n = 7)

Parameters	5PRE	1POST	10POST	20POST	30POST	60POST	120POST	360POST	12hPOST	24hPOST
RR (mpm)										
GI	16.5±4.0	21.7±1.9	24.8±2.2	15.5±2.8	19.7±2.7	19.6±2.5	19.2±2.0	15.5±3.1	16.2±4.1	16.5±3.8
GII	16.7±3.1	24.8±1.8	25.4±2.9	15.8±2.9	18.8±3.0	17.8±3.5	17.1±3.1	16.1±3.5	16.5±3.3	16.1±3.0
HR (bpm)										
GI	92.4±25.6	100.0±20.0	88.0±12.8	85.1±15.0	89.4±10.2	89.2±10.0	88.8±10.2	90.3±8.2	92.8±25.3	92.1±24.8
GII	93.1±18.3	79.4±19.9	83.1±26.6	78.5±14.5	84.5±17.6	84.2±17.4	84.8±17.7	92.6±16.3	93.3±18.0	93.1±17.8
BT (º C)										
GI	39.1±0.2	38.7±0.4	38.7±0.4	38.6±0.3	38.6±0.3	38.2±0.1	38.1±0.1	38.0±0.2	38.6±0.1	38.5±0.2
GII	38.8±0.5	38.4±0.9	38.3±0.8	38.1±0.2	38.6±0.3	38.4±0.2	38.3±0.4	38.2±0.2	38.5±0.4	38.4±0.3
SBP (mmHg)										
GI	120.0±10.7	120.8±10.8	121.1±10.5	119.2±9.5	130.7±20.8	130.7±20.9	121.6±10.2	120.6±10.1	121.0±10.1	119.1±9.2
GII	130.4±10.9	120.8±20.4	120.6±20.3	120.1±20.1	120.7±10.7	120.8±10.3	130.8±10.8	131.1±10.6	131.4±10.8	128.4±8.6
cTnI (ng/mL)										
GI	0.01±0.0	0.01±0.00	-	-	0.01±0.0	0.01±0.0	0.01±0.01	0.02±0.01	0.01±0.0	0.01±0.0
GII	0.02±0.0	0.02±0.01	-	-	0.02±0.01	0.03±0.01	0.02±0.0	0.01±0.01	0.02±0.01	0.02±0.01

*No differences were identified between groups and time-points. RR – Respiratory Rate; HR- Heart Rate; BT – BodyTemperature; SBP - Systolic Blood Pressure; cTnI - Cardiac Troponin I; 5PRE – 5-min before WBV; 1POST – 1-min after WBV; 10POST – 10-min after WBV; 20POST – 20-min after WBV; 30POST – 30-min after WBV; 60POST – 60-min after WBV; 120POST – 120-min after WBV; 360POST – 360-min after WBV, 12hPOST – 12 h after WBV; 24hPOST – 24 h after WBV.

Table 2. Mean and standard deviation (Mean ± SD) of IVSd, LVIDd, LVFWd, IVSs, LVIDs, LVFWs values at 5PRE, 1POST, 12hPOST, and 24POST in young (GI, n = 7) and aged dogs (GII, n = 7)

Parameters	5PRE	1POST	12hPOST	24hPOST
IVSd (cm)				
GI	$0.9\pm0.2^{A,a}$	$0.9\pm0.1^{A,a}$	$0.8\pm0.2^{A,a}$	$0.9\pm0.1^{A,a}$
GII	$1.2\pm0.2^{A,b}$	$1.2\pm0.2^{A,a}$	$1.1\pm0.2^{A,a}$	$1.2\pm0.1^{A,a}$
LVIDd (cm)				
GI	$3.2\pm0.7^{A,a}$	$3.0\pm0.7^{A,a}$	$3.1\pm0.8^{A,a}$	$3.2\pm0.6^{A,a}$
GII	$3.5\pm0.3^{A,a}$	$3.5\pm0.3^{A,a}$	$3.4\pm0.4^{A,a}$	$3.4\pm0.1^{A,a}$
LVFWd (cm)				
GI	0.8 ± 0.1^{a}	NaN±NA	NaN±NA	NaN±NA
GII	0.9 ± 0.1^{b}	NaN±NA	NaN±NA	NaN±NA
IVSs (cm)				
GI	$1.3\pm0.2^{A,a}$	$1.2\pm0.3^{A,a}$	$1.2\pm0.1^{A,a}$	$1.3\pm0.1^{A,a}$
GII	$1.5\pm0.2^{A,a}$	$1.5\pm0.2^{A,a}$	$1.4\pm0.3^{A,a}$	$1.4\pm0.1^{A,a}$
LVIDs (cm)				
GI	$2.0\pm0.4^{A,a}$	$2.0\pm0.4^{A,a}$	$2.0\pm0.3^{A,a}$	$2.0\pm0.3^{A,a}$
GII	$2.1\pm0.3^{A,a}$	$2.1\pm0.3^{A,a}$	$2.1\pm0.2^{A,a}$	$2.1\pm0.4^{A,a}$
LVFWs (cm)				
GI	$1.1\pm0.2^{A,a}$	$1.1\pm0.2^{A,a}$	$1.1\pm0.1^{A,a}$	$1.2\pm0.2^{A,a}$
GII	$1.3\pm0.2^{A,a}$	$1.2\pm0.1^{A,a}$	$1.3\pm0.1^{A,a}$	$1.3\pm0.1^{A,a}$

NaN±NA – Not performed. Means followed by different lower-case letters in the same column were significantly different by the Mann-Whitney test (p <0.05). IVSd – Thickness of the interventricular septum; LVIDd - Internal diameter of the left ventricle; LVFWd - Left ventricular wall; IVSs - Thickness of the interventricular septum; LVIDs - Internal diameter of the left ventricle; LVFWs - Thickness of the left ventricular wall; 5PRE – 5-min before WBV; 1POST – 1-min after WBV; 12hPOST – 12 h after WBV; 24hPOST – 24 h after WBV.

Table 3. Mean and standard deviation (Mean ± SD) of LA, Ao, LA/Ao, PFV, PPG, LVSF, and EF values at 5PRE, 1POST, 12hPOST and 24POST in young (GI, n = 7) and aged dogs (GII, n = 7)

Parameters	5PRE	1POST	12hPOST	24hPOST
LA (cm)				
GI	2.4±0.3^{Aa}	2.4±0.4^{Aa}	2.3±0.4^{Aa}	2.4±0.3^{Aa}
GII	3.1±0.2^{Ab}	3.0±0.2^{Bb}	3.1±0.1^{Aa}	3.0±0.3^{Aa}
Ao (cm)				
GI	2.0±0.2^{Aa}	1.7±0.2^{Aa}	1.9±0.2^{Aa}	2.0±0.3^{Aa}
GII	2.0±0.2^{Aa}	2.0±0.2^{Aa}	1.8±0.3^{Aa}	2.0±0.1^{Aa}
LA/Ao				
GI	1.3±0.1^{Aa}	1.4±0.1^{Aa}	1.4±0.2^{Aa}	1.3±0.2^{Aa}
GII	1.5±0.2^{Aa}	1.4±0.2^{Aa}	1.4±0.1^{Aa}	1.3±0.3^{Aa}
PFV (m/s)				
GI	0.8±0.1^{Aa}	0.8±0.2^{Aa}	0.8±0.1^{Aa}	0.8±0.2 ^{Aa}
GII	1.0±0.2^{Aa}	1.0±0.1^{Aa}	1.0±0.^{Aa}	1.0±0.1^{Aa}
PPG (mmHg)				
GI	3.0±1.0^{Aa}	3.0±1.1^{Aa}	3.0±1.1^{Aa}	3.0±1.1^{Aa}
GII	3.2±1.1^{Aa}	3.3±1.1^{Aa}	3.2±1.0^{Aa}	3.2±1.2^{Aa}
LVSF (%)				
GI	38.0±4.4^{Aa}	37.8±4.2^{Aa}	37.9±4.2^{Aa}	38.0±4.3^{Aa}
GII	38.6±5.0^{Aa}	39.7±3.7^{Aa}	39.6±3.5^{Aa}	38.5±4.9^{Aa}
EF (%)				
GI	70.0±4.6^{Aa}	70.3±4.6^{Aa}	70.3±4.4^{Aa}	70.1±4.5^{Aa}
GII	70.3±6.0^{Aa}	71.9±2.0^{Aa}	71.7±2.2^{Aa}	70.2±5.8^{Aa}

NaN±NA – Not performed. Means followed by different capital letters on the same line were significantly different by the mixed model test (P<0.05). Means followed by different lower-case letters in the same column were significantly different by the Mann-Whitney test ($p < 0.05$). LA - Diameter of the left atrium; Ao – Diameter of Aorta during diastole; LA/Ao - Left atrium/aorta ratio; PFV - Pulmonary flow velocity; PPG - Pulmonary pressure gradient), LVSF - Left ventricle-shortening fraction; 5PRE – 5-min before WBV; 1POST – 1-min after WBV; 12hPOST – 12 h after WBV; 24hPOST – 24 h after WBV.

Table 4. Mean and standard deviation (Mean ± SD) of Vel E_mitr, TDEm, VelA_mitr, TRIV, E_A, E_sep_VE and A_sep_VE values at 5PRE, 1POST, 12hPOST and 24POST in young (GI, n = 7) and aged dogs (GII, n = 7)

Parameters	5PRE	1POST	12hPOST	24hPOST
VelE_mitr (m/s)				
GI	0.7±0.1	0.7±0.1	0.7±0.1	0.7±0.1
GII	0.7±0.2	0.7±0.1	0.7±0.1	0.7±0.2
TDEm				
GI	108.6±13.2	100.1±14.3	100.6±13.4	108.5±14.0
GII	107.1±17.4	110.1±7.0	109.1±7.4	108.2±17.2
VelA_mitr(m/s)				
GI	0.6±0.1	0.66±0.1	0.7±0.1	0.61±0.2
GII	0.6±0.1	0.66±0.1	0.7±0.1	0.62±0.1
TRIV (s)				
GI	51.6±2.1	50.7±4.4	51.0±2.0	51.5±2.0
GII	52.4±5.0	54.0±4.0	54.2±3.9	52.1±4.8
E_A				
GI	1.3±0.3	1.3±0.3	1.3±0.2	1.3±0.1
GII	1.2±0.2	1.2±0.2	1.2±0.3	1.2±0.3
E_sep_VE (m/s)				
GI	0.6±0.1	0.6±0.1	0.6±0.2	0.6±0.1
GII	0.6±0.1	0.6±0.1	0.6±0.2	0.6±0.1
A_sep_VE (m/s)				
GI	0.6±0.1	0.6±0.1	0.6±0.1	0.6±0.2
GII	0.6±0.1	0.6±0.1	0.6±0.1	0.6±0.0

*No differences were identified between groups and time-points. Vel E_mitr – Mitral flow of E-wave; TDEm - deceleration time of E-wave; VelA_mitr – Mitral flow of A-wave; TRIV - Isovolumetric relaxation time; E_A - Ratio of the E-wave and A-wave; E_sep_VE - Fast and slow ventricular filling time; A_sep_VE - Atrial contraction. ; 5PRE – 5-min before WBV; 1POST – 1-min after WBV; 12hPOST – 12 h after WBV; 24hPOST – 24 h after WBV.

Ambulatory electrocardiographic (Holter): Significant variations were observed in HRmin: GI < GII ($p = 0.0370$) at 5PRE WBV (5PRE) and 12hPOST. Group II showed a decrease ($p = 0.0370$) in HRmin values between 5PRE and 12hPOST, and 1POST and 12hPOST (Tab. 5). HRaver values showed statistical variation: GI < GII ($p = 0.0120$) at 360POST and 12hPOST. Decrease ($p = 0.0120$) was observed in the same parameter in GII between the following time

points: 5PRE > 12hPOST, 1POST > 12hPOST, 120 (120POST) > 360POST, 120POST > 12hPOST (Tab. 5). Differences GI < GII (p < 0.01) in HRmax values was observed at 5-min before the dogs were submitted to WBV.

NNm showed significant variation: GI < GII (p = 0.0370) at 360POST and 12hPOST (Tab. 5). The values of NNs showed a difference: GI > GII (p = 0.0295) at 12hPOST; and GI demonstrated increased values (P = 0.0295) between 120POST and 360POST (Tab. 5). The values of SDNN had variation between both groups as following: GI < GII (p < 0.01) at 12 h after the WBV session. GI presented an increase (p < 0.01) in the same parameter between 120POST and 360POST, and a decrease (p < 0.01) among the following time points: 120POST > 4hPOST, 12hPOST > 24hPOST. GII showed increase (P < 0.01) in the following time-points: 5PRE < 360POST, 5PRE < 12hPOST, 1POST < 360POST, 120POST < 360POST, 360POST < 4hPOST (Tab. 5). Variations of SDANN values were observed: GI < GII (p < 0.01) at 24 h after the WBV session. On the other hand, GII showed increase (p < 0.01) at 5PR < 360POST, 5PRE < 24hPOST (Tab. 5). rMSSD values demonstred significant differences in GI < GII (p < 0.01) at 12 h after the WBV session. Finally, group GI presented an increase (p < 0.01) in the following time-points: 120POST < 360POST, and an increase (p = 0.0208) in pNN > 50 values: GI < GII at 120 and 360 min after the WBV session (Tab. 5).

Table 5. Mean and standard deviation (Mean ± SD) of HRmin, HRaver, HRmaxim, NNm, SDNNIDX, NNs, SDNN, SDANN, rMSSD, pNN>50 values at six different time points: 5PRE, 1POST, 120POST, 360POST, 12hPOST, and 24hPOST in young (GI, n = 7) and aged dogs (GII, n = 7)

Parameters	5PRE	1POST)	120POST	360POST	12hPOST	24hPOST
HR_{min}						
GI	74.9±17.9^{Aa}	69.9±12.7^{Aa}	57.1±19.4^{Aa}	52.3±6.4^{Aa}	52.3±6.4^{Aa}	59.4±29.5^{Aa}
GII	69.0±12.0^{ABb}	67.9±19.2^{ACa}	61.0±21.3^{ACDa}	44.1±14.8^{ADa}	38.8±12.4^{DEb}	59.3±29.7^{ADa}
HRaver						
GI	121.9±31.1^{Aa}	117.4±20.7^{Aa}	96.4±28.0^{Aa}	91.0±16.0^{Aa}	91.0±16.0^{Aa}	110.3±34.1^{Aa}
GII	105.9±23.3^{AEa}	104.3±24.1^{ACEa}	124.4±21.4^{ACa*}	76.3±24.7^{DEb}	69.1±17.1^{BDb*}	99.4±32.6^{ADa}
HRmaxim						
GI	196.1±52.8^{Aa}	231.7±14.8^{Aa}	177.7±42.3^{Aa}	197.6±27.3^{Aa}	197.6±27.3^{Aa}	188.1±39.8^{Aa}
GII	206.3±57.5^{Ab}	190.1±45.4^{Aa}	235.3±19.3^{Aa}	182.0±47.4^{Aa}	171.0±52.0^{Aa}	199.0±70.7^{Aa}
NNm						
GI	568.6 ± 122.0^{Aa}	541.0 ± 72.7^{Aa}	535.1 ± 107.1^{Aa}	698.4 ±136.2^{Aa}	704.7 ± 117.8^{Aa}	624.5 ± 176.9^{Aa}
GII	631.4 ± 119.6^{Aa}	620.1 ± 147.5^{Aa}	550.0 ± 93.2^{Aa}	882.6 ±188.6^{Ab}	1030.1 ± 239.7^{Ab}	688.6 ± 200.8^{Aa}
SDNNIDX						
GI	135.6 ± 79.9^{ABa}	117.3 ± 47.6^{ABa}	91.0 ± 33.6^{Ba}	235.4 ± 55.5^{Aa}	238.1 ± 77.5^{ABa}	162.7 ± 93.8^{ABa}
GII	110.7 ± 26.4^{Aa}	101.3 ± 46.3^{Aa}	110.6 ± 70.3^{Aa}	327.6 ± 108.6^{Aa}	413.9 ± 118.1^{Ab}	219.5 ± 124.1^{Aa}
NNs						
GI	323.4 ± 208.1^{Aa}	585.74 ± 148.4^{Aa}	570.71 ± 144.30^{Aa}	463.1 ± 736.4^{Aa}	485.1 ± 598.4^{Aa}	238.5 ± 129.8^{Aa}
GII	250.9 ± 209.1^{Aa}	496.90 ± 223.7^{Aa}	129.7 ± 62.5^{Aa}	407.1± 1026.9^{Aa}	334.3 ± 672.1^{Ab}	366.2 ± 188.7^{Aa}
SDNN						
GI	159.6 ±100.2^{ABCDFGa}	113.0 ± 36.7^{AGa}	227.4 ± 88.7^{CEGa}	255.6 ± 69.7^{ABDa}	255.6 ± 69.7^{BGa}	178.0 ± 89.7^{AFa}
GII	124.3 ± 22.2^{Aa}	130.4 ± 58.0^{Aa}	172.4 ± 73.0^{Aa}	364.4 ± 131.1^{Ba}	443.3 ± 133.6^{BCb}	239.3 ± 135.3^{ADa}
SDANN						
GI	50.0±27.5^{Aa}	51.3±12.5^{Aa}	59.9±19.4^{Aa}	58.3±24.0^{Aa}	74.1±34.3^{Aa}	57.4±48.0^{Aa}
GII	26.5±15.3^{Aa}	62.0±43.7^{Aa}	110.6±70.3^{ABCa}	160.0±102.0^{Ba}	118.4±64.0^{ABa}	165.5±46.4^{BCb}
rMSSD						
GI	184.0 ±158.2^{ABa}	131.1 ± 82.6^{ABa}	92.3 ± 48.1^{Aa}	288.1 ± 75.4^{Ba}	282.4 ± 85.6^{ABa}	206.8 ± 196.9^{ABa}
GII	122.8 ± 25.2^{Aa}	116.8 ± 61.4^{Aa}	136.3 ± 69.4^{Aa}	466.1 ± 136.9^{Aa}	533.0 ± 124.0^{Ab}	250.1 ± 176.6^{Aa}
pNN>50						
GI	38.2 ± 32.7^{ABa}	36.3 ± 20.0^{ABa}	29.9 ± 19.1^{Aa}	69.8 ± 6.6^{Ba}	63.0 ± 14.6^{ABa}	44.4 ± 35.0^{ABa}
GII	33.3 ± 14.9^{ABa}	31.5 ± 17.7^{ABa}	26.0 ± 11.3^{Aa}	70.9 ± 16.7^{Ba}	79.0 ± 10.5^{ABa}	41.0 ± 21.6^{ABa}

Means followed by different capital letters on the same line were significantly different by the mixed model test (P <0.05). Means followed by different lower-case letters in the same column were significantly different by the Mann-Whitney test (p <0.05). HRmin – Minimum heart rate; HRaver – Average heart rate; HRmaxim – Maximum heart rate; NNm - Interval between two consecutive N-waves of the entire record; SDNNIDX - Normal RR intervals every 5-min, NNs - Normal RR intervals; SDNN - RR wave intervals; SDANN - Interval mean NN-wave obtained every 5-min; rMSSD - Differences between adjacent normal RR or NN intervals; pNN>50 - NN intervals with a difference in duration greater than 50 milliseconds; 5PRE – 5-min before WBV; 1POST – 1-min after WBV; 12hPOST – 12 h after WBV; 24hPOST – 24 h after WBV.

DISCUSSION

This study evaluated the short-term effects of WBV in clinical, cardiac, and ambulatory electrocardiographic (Holter) parameters of healthy young and aged neutered male non-athletic crossbreed dogs. The hypothesis that a single session of WBV with frequencies of 30 and 50Hz, for 15-min, would not induce variations on these parameters was proven since the values that showed significant differences remained within the reference values.

Authors that used the same vibrating platforms and WBV protocol in dogs did not identify significant alteration in renal (Freire *et al*., 2015)

and common carotid resistive index (Nagai *et al*., 2020), or hemogasometric values (Silva *et al*., 2020). On the other hand, Santos *et al.* (2019) identified an increase in the femoral resistive index and a decrease in femoral muscle thermography values by using digital infrared thermal imaging in adult male Beagle dogs submitted to WBV (one session) for uninterrupted five days. This fact was related to the absence of 24 h rest between the sessions. Aoyama *et al*. (2019) did not identify significant changes in heart rate, systolic blood pressure, and diastolic blood pressure during WBV training in elderly patients with cardiovascular diseases who underwent static resistance training on the WBV vertical platform with a protocol of 30 Hz (3-mm peak-to-peak amplitude, 30 seconds).

Neutered male dogs were used to avoid hormonal influences. A study by Schaible *et al*. (1984) identified the relationship between testosterone and ventricular function in gonadectomized rats. Schaible *et al*. (1984) observed a reduction in cardiac output, systolic blood pressure, ejection fraction; and these changes were associated with a reduction in the activity of myosin ATPase attributed to testosterone deficiency. Alternatively, interactions with unfamiliar humans were avoided using dogs who were familiarized with the evaluators and prevented stress conditions. RR, HR, BT, and SBP values did not show variations between groups and time-points, similar to studies in human patients (Button *et al*., 2007, Otsubi *et al*., 2008). However, long-term WBV increases HR and SBP by 30 and 15%, respectively (Rittweger *et al*., 2000).

The absence of variations in cTnI values in this study was related to the absence of myocardial cell damage. The mechanism regarding the increase of cTnI after exercise is unknown (Harper *et al*., 2020), but a direct relationship between exercise intensity and myocardial damage was reported in the literature (Lee *et al*., 2012; Rossi *et al*., 2019). Increases were observed in IVSd, LVFWd, and LA values but remained within the reference values. Gehlen *et al.* (2006) conducted a study regarding the influence of fitness on left ventricular function parameters in healthy warmblood horses, trained and non-trained, at rest and immediately after high-speed treadmill exercise. In the same study, was observed differences in echocardiographic parameters (most diastolic) between rest and exercise in trained horses, which the left ventricular diameter at the apex cordis, left ventricular, free wall at the papillary muscle level, left ventricular volume and stroke volume, as well as fractional shortening (at the apex cordis and papillary muscle level), were increased in trained horses in the rest period.

Variations were observed in HRmin, HRaver, HRmax, SDNN, SDANN, and rMSSD values but they remained within the reference values. The increase in SDNN, NNs, SDANN, and rMSSD values, and decrease of all HR along the time-points were expected and were considered a beneficial effect of WBV in healthy young and aged dogs due to autonomic nervous system variations, corroborated with literature (Spier & Meurs 2004). Similarly, a study performed in elderly human patients demonstrated an increase in SDNN, rMSSD, and pNN50 after a single session of WBV (orbital vibration) (20 Hz, 6 mm displacement, 10min).

A decrease in HRmin, HRaver, and HRmax values was associated with vagal control of the heart during the rest period (Barretto *et al*., 2013). Similarly, Cavalcanti *et al*. (2009) evaluated ambulatory electrocardiographic (Holter) in young German shepherd dogs during physical exercise and at rest and was identified a decrease in all HR values during the night and rest period.

This study had limitations that included a reduced number of animals in each group due to the strict inclusion criteria, therefore future studies should be conducted with a higher number of animals.

The clinical significance of this study was related to using WBV as a resistance exercise in dogs. However, the cardiovascular measurements performed in this study can indirectly reflect the muscular system since WBV can promote muscle contractions and regional blood supply due to vasodilation and, consequently, an increase in muscle mass.

CONCLUSION

A single session of WBV at frequencies of 30 and 50 Hz during 15-min using a vibrating platform that delivered a vortex wave circulation does not induce changes in clinical, cardiac, and ambulatory electrocardiographic (Holter) parameters in healthy young and aged neutered male non-athletic crossbreed dogs.

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