

# Effects of Short-Period Whole-Body Vibration of 20 Hz on Selected Blood Biomarkers in Wistar Rats

Milena de Oliveira Bravo Monteiro<sup>1, 2</sup>, Danúbia da Cunha de Sá-Caputo<sup>3</sup>,  
Fernanda Santos do Carmo<sup>1, 2</sup>, Raquel Mattos Bernardo<sup>2</sup>, Raphaelle Pacheco<sup>2</sup>,  
Adriano Arnóbio<sup>1</sup>, Carlos Alberto Sampaio Guimarães<sup>2</sup>, Luciana Camargo Bernardo<sup>2</sup>,  
Sebastião David Santos-Filho<sup>2</sup>, Nasser Ribeiro Asad<sup>2</sup>, Marianne Unger<sup>4</sup>,  
Pedro Jesus Marin<sup>5</sup>, and Mario Bernardo-Filho<sup>2</sup>

<sup>1</sup>*Programa de Pós-Graduação em Ciências Médicas, Universidade do Estado do Rio de Janeiro, Rio de Janeiro, RJ, Brasil*

<sup>2</sup>*Departamento de Biofísica e Biometria, Instituto de Biologia Roberto Alcântara Gomes, Universidade do Estado do Rio de Janeiro, Rio de Janeiro, RJ, Brasil*

<sup>3</sup>*Mestrado Profissional em Saúde, Medicina Laboratorial e Tecnologia Forense, Rio de Janeiro, RJ, Brasil*

<sup>4</sup>*Division of Physiotherapy, Faculty of Medicine and Health Sciences, Stellenbosch University, Cape Town, South Africa*

and

<sup>5</sup>*Laboratorio de Fisiología, Universidad Europea Miguel de Cervantes, Valladolid, Spain*

## Abstract

There is a growing interest in the use of vibration generated by oscillating/vibratory platforms – also known as whole-body vibration (WBV) – for achieving therapeutic, preventative and/or physical performance goals. This study investigated the effects of vibration generated by an oscillating platform on the concentration of blood biomarkers in rats. Wistar rats ( $n = 8$ ) were divided into 2 groups, sedated and individually positioned on an oscillating platform. The experimental group (EG) was subjected to vibrations of 20 Hz for one min per day for one week while the control group (CG) experienced no vibration. Samples of heparinized whole blood were drawn by cardiac puncture for biochemical analysis. Concentrations of total cholesterol, triglycerides, HDL, LDL, VLDL, glucose, CK, albumin, alkaline phosphates, TGP, TGO,  $\gamma$ GT, lipase, amylase, urea and creatinine were determined. White blood cell count and a platelet hemogram were also performed. Following seven sessions of exposure to the vibration, a significant ( $P < 0.05$ ) reduction in  $\gamma$ GT, VLDL and leukocytes was found. A weekly 1-min/day exposure of 20 Hz vibration can be shown to alter the concentrations of selected blood biomarkers in rats. The action mechanism associated with these effects seems highly complex, but the findings might contribute to the understanding of these mechanisms related to the exposure to 20 Hz vibration.

**Key Words:** biochemical analysis, blood cells, frequency, oscillating platform, vibration, whole body vibration exercise

## Introduction

There is a growing interest in the use of vibra-

tion generated by oscillating/vibratory platforms – also known as whole-body vibration (WBV) – for achieving therapeutic, preventative and/or physical

Corresponding author: Mario Bernardo-Filho, Departamento de Biofísica e Biometria, Instituto de Biologia Roberto Alcântara Gomes, Universidade do Estado do Rio de Janeiro, 20551-030, Av. 28 de setembro, 87, fundos, 4º andar, Rio de Janeiro, RJ, Brasil, phone: +55 21 2868 8332, móbile: 55 21 992908935, E-mail: bernardofilhom@gmail.com

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performance goals (12, 37, 39). Vibration is a mechanical stimulus characterized by an oscillatory motion the intensity of which can be controlled by adjusting the frequency, the peak to peak displacement and the time of exposure (38). Moreover, the force that acts on the participant on the oscillating/vibratory platform depends on the mass and on the acceleration in body due to the exposition to the vibration (force = mass  $\times$  acceleration) (9, 36, 37, 39).

During WBV training, the participant normally stands on a platform while maintaining a static position, or is performing dynamic exercise, and the human body is accelerated causing a reactive force by and within the body (9, 20, 39). Investigations have well documented the effects of vibration on neuromuscular structures (12, 32, 39, 40). Exposure to WBV has been shown to increase lower limb force production and to improve gait (15, 23, 24, 36). These effects have been reported in various populations including healthy individuals and persons with neuromuscular conditions such as Parkinson's disease, stroke, multiple sclerosis and cerebral palsy (36, 39). Significant increase in bone mineral density has been reported in both adults and children participating in various WBV treatment protocols (36). One study has also shown WBV to be effective in reducing delayed onset muscle soreness and stiffness following running in untrained athlete (35); and another study in patients with neurological disorders a temporary decrease in spasticity was also been reported (1, 19). Investigation of a procedure combining vibration generated in an oscillating platform and acupuncture with needles in a patient with blepharitis has been also described (4).

In experimental models, research has shown that the vibration generated by oscillating platforms is capable of interfering with osmotic fragility of red blood cells when blood samples are submitted to vibrations (28). Study with Wistar rats demonstrated that exposure of vibration (20 Hz, 1 min, acute effect) to animals could alter the uptake of a radiopharmaceutical ( $^{99m}$  technetium-methylenediphosphonic acid) in organs such as the stomach, bowel, kidneys, urinary bladder and prostate (33). Prisby *et al.* (36) reported some investigations that suggested that vibration also has effects on the bone. Christiansen and Silva (11) reported that increasing accelerations of vibrations (45 Hz for 15 min/day, seven days/week for five weeks) enhanced trabecular bone volume in a non-dose-dependent fashion as assessed by histo-morphometry in the proximal tibia of adult mice. Rubin *et al.* (41) observed a significant increase in femoral trabecular bone mass in adult ewes following vibration (30 Hz, 7 for 20 min per day for 5 days a week, one year), as compared to controls. Garman *et al.* (16) described that trabecular bone formation rate to bone surface

ratio (BFR/BS) and mineralizing surface to bone surface ratio (MS/BS) were enhanced in female mice following vibration (5 days/week, 10 min/day, 45 Hz, three weeks).

The determination of the concentration of biomarkers can aid to understand the action mechanisms related to some biological responses. Lin *et al.* (25) evaluated the relationship between expression of LGR8, VEGF, MMP-2, MMP-9, fascin-1 and cortactin with clinicopathological parameters in hepatocellular carcinoma. The tumor cells showed significant expression of LGR8, VEGF, MMP-9, fascin-1 and cortactin, but not of MMP-2. Cheng *et al.* (10) have verified over-expression of several biomarkers in gastric cancer. Studies investigating the effects of vibration in human beings have also reported biological responses with alterations in some biomarkers. Hormonal changes such as an increase in testosterone and growth hormone and a decrease in cortisol was found (5, 8, 22, 43); changes in fatty acid concentrations (18) and blood glucose levels (3) have been reported resulting from participating in vibration efficacy trials. An increase of epinephrine and norepinephrine was also reported (14). Naghii *et al.* (30) have verified in male rats submitted to vibrations (one to three 5 min cycles of WBV 10-50 Hz, four sessions in the first week and increased gradually to 45 min until day 24 with three sessions per week; followed by 60 min per set for the next 20 sessions until 8 weeks) significant differences in plasma levels of creatine kinase (CK), estradiol (E2) and interleukin-6 (IL-6) between the vibration and control groups. The mean vitamin D level was 15% higher and IL-6 level was 32% higher in the group exposed to vibration. Similarly Naghii and Hedayat (31) reported a significant increase in plasma levels of xanthine oxidase due to vibrations (one-three 5-min cycles of WBV 10-50 Hz, four sessions in the first week and increased gradually to 45 min until day 24 with three sessions per week, followed by 60 min per set for the next 20 sessions until the end of the eight weeks). Pawlak *et al.* (32) subjected two groups of Wistar rats to WBV (five days a week, frequency of 50 Hz, for 3 months and 6 months, exposed to a single session of daily vibration, every session included four bouts lasting 30 s, separated by 1 min rest intervals). Blood was collected and red and white blood cells, lymphocytes, monocytes, granulocytes, hemoglobin and hematocrit as well as IL-1b, IL-10, IL-6 and vascular endothelial growth factor levels were determined. No significant differences between the two experimental groups and the control group were found for either total blood counts or selected immunological parameters. The apparent discrepancy between studies regarding the effects of vibration on biomarkers may be attributable to the fact that the studies used different populations, from rats to human beings.

The various protocols have contributed to different applications of the WBV (1, 17, 19, 26, 46). Marin *et al.* (27) suggested that change in peak to peak displacement or in frequency of the vibration can affect the rate of change of the WBV acting on an individual. A protocol using a low frequency (5 Hz) showed a potentially desirable effect in delay-onset muscle soreness (29). Haas *et al.* (19) have shown that a protocol with vibration with 6 Hz improved the tremor and rigidity scores by 25% and 24%, respectively, in patients with Parkinson disease. Ahlborg *et al.* (1) have demonstrated that 8-week intervention of WBV training with frequencies 25–40 Hz can increase muscle strength without negative effect on spasticity in adults with cerebral palsy.

Habitual physical activity (HPA) has many benefits and has been shown to reduce the risk of coronary heart disease, stroke, colon cancer and mortality from all causes (6, 7, 13). In patients with diabetes mellitus, HPA has been shown to affect the metabolism of glucose and other intermediate substrates (3, 13). As exercise is a type of physical activity and as vibration can promote WBV exercise, the vibration generated in oscillating/vibratory may also be effective in promoting recovery following injury and disease (36, 39, 46).

Putting together all the findings reported in the literature about the WBV exercises, it is clear that more research is needed to better understand the specific therapeutic potential of vibrations generated in the oscillating/vibratory platform (39). Moreover, many of the effects reported (9, 36, 39) may be due to indirect action of the vibration that can result in changes in concentrations in some biomarkers in blood (36) in response to action of the vibration on the endocrine system. The purpose of this preliminary set of studies was to determine the effects of 20 Hz vibration generated by an oscillating platform on several biochemical blood markers in Wistar rats.

## Materials and Methods

### *Ethical Approval and Animal Conditions*

All the experimental procedures followed the Ethical Guidelines of the *Instituto de Biologia Roberto Alcântara Gomes, Universidade do Estado do Rio de Janeiro*, Brazil, with the protocol number CEA/024/2009. A veterinary physician was always present during the experiment. Eight Wistar rats (male, 3–4 months of age, weighing between 260–370 g) were used in this study. The rats were kept under the same environmental conditions ( $25 \pm 2^\circ\text{C}$ , 12 h of light/dark cycle), given water *ad libitum* and fed a normal diet.

### *Characteristics of the Oscillating Platform*

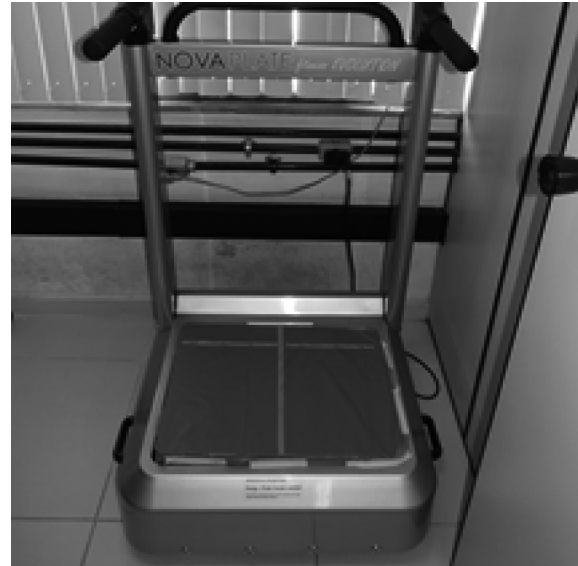


Fig. 1. The oscillating platform used in the experiments.

The platform used in the study was an oscillating system (Novaplate fitness evolution, DAF, Produtos Hospitalares Ltda, São Paulo, Brazil) with reciprocating vertical displacements on the left and right side of a fulcrum (9, 39). It is a side-alternating vibration (38) device working as a teeterboard (28 cm  $\times$  58 cm) with amplitude of 0 mm in the center of the platform up to the maximum in the edge that was 7.07 mm, as it was measured as informed by the manufacturer (Fig. 1).

### *Study Design*

As reported by several authors (3, 5, 9, 18, 22, 43), WBV can exert influence in various pathways of the metabolism of various organs. A group-controlled experimental design was used to evaluate the effects of vibration on the following biomarkers: total cholesterol, triglycerides, HDL (high density lipoprotein), LDL (low density lipoprotein), VLDL (very low density lipoprotein), glucose, CK, albumin, alkaline phosphatase, TGP (glutamic pyruvic transaminase), TGO (glutamic oxaloacetic transaminase),  $\gamma$ GT (gamma glutamyl transpeptidase), lipase, amylase, urea and creatinine. The effect of vibration on the number of white blood cells and platelets, and in the percentage of neutrophils, lymphocytes, monocytes, eosinophils and basophils were also investigated.

### *Experimental Procedure*

The animals (eight Wistar rats) were sedated with sodium thiopental, 50 mg/kg body weight, and divided into two groups of four rats each. They were positioned one on each side of the platform and had its

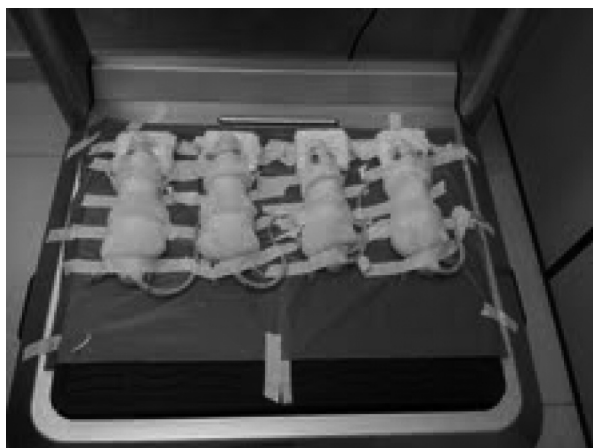


Fig. 2. Rats fixed by tape on the base of the oscillating platform.

head fixed with tape resting on a makeshift gauze pillow as shown in Fig. 2. This procedure was done to protect the head of the animal to avoid contact with the base of the platform. Moreover, in this condition, the whole body of the animals received the same intensity of the vibration energy, except the head. The animals ( $n = 4$ ) of the experimental group (EG) were submitted to 20 Hz for one min per day for one week for seven sessions, with a total of seven minutes of treatment. Each animal was identified and always put in the same place in the teeterboard of the platform in the different sessions. As shown in Fig. 2, considering the width of the animals, a small difference in peak-to-peak displacement was observed in the rats in each side of the teeterboard. The lasting WBV time of the animals in each session presents an important variation among the authors (21, 30). In this investigation, a total time of WBV per week of seven min was used, which was similar to the study of Pawlak *et al.* (32) which was ten min per week. Following Yang *et al.* (47) in another study, four animals were designated to be in the control group (CG) and they were not submitted to the vibration.

#### Blood Samples

As all animals were under controlled conditions since they were born, no baseline testing was done. Immediately after completing the one-week intervention protocol, heparinized (4% heparin) whole blood from both groups was drawn (4 ml per animal) by cardiac puncture under anesthesia. A veterinary physician rapidly withdrew the blood of the animals at about 7 a.m. The concentrations of selected biomarkers were then measured in a clinical laboratory of the *Universidade do Estado do Rio de Janeiro*. The determinations were performed in an automated equipment (COBAS INTEGRA 400 plus, Roche,

Basel, Switzerland).

#### Statistical Analysis

Although statistical analysis was used to determine differences between two groups of animals (CG and EG), as an option, ANOVA variance analysis (one-way) followed by the Bonferroni test was performed. As these rats were a homogenous group, bred and exposed to the same environmental and stress conditions, the post-test measurements allowed for the assumption that changes in concentrations detected in the blood samples were attributed to the effect of the intervention. Data are presented as mean  $\pm$  standard deviation (SD). Statistical significance was accepted at  $P < 0.05$ . Absolute effect sizes,  $d$ , were analyzed to determine the magnitude of an effect between groups independent of sample size. *Small* effect sizes are considered  $d < 0.2$ , *moderate* effect sizes are  $0.2 < d < 0.8$ , and *large* effects sizes are  $d \geq 0.8$  (37).

#### Results

Our results showed that exposure to vibration had led to varied outcome on the concentration of biomarkers measured in this cohort of rats. The effects of exposure to 20 Hz vibration are summarized in Table 1 for biomarkers related to the liver function. The  $\gamma$ GT concentration was significantly reduced ( $P < 0.05$ ) in the EG compared to the CG with a large effect size ( $d = 1.80$ ). No significant difference was found in the concentration of the other blood biomarkers.

The effects of 20 Hz vibration exposure on the levels of biomarkers related to lipid metabolism are shown in Table 2. The VLDL concentration decreased significantly ( $P < 0.05$ ) in the EG with a large effect size ( $d = 1.60$ ). No significant difference was found in the concentration of the other blood biomarkers.

For measures of biomarkers related to kidney and pancreas functions, no significant differences between the EG and CG were found (Table 3) as a result of the exposure to 20 Hz vibration.

The effects of 20 Hz vibration on the concentration of blood cells are shown in Table 4. The number of leukocytes increased significantly due to the exposure to the vibration ( $P < 0.05$ ) compared to the CG with a moderate effect size ( $d = 0.33$ ). No significant difference was found in the concentration of the other blood cells.

#### Discussion

The results of this study confirm that exposure to vibration at 20 Hz did affect the concentration of



**Table 1. Effects of 20 Hz vibration on plasma biomarkers related to the liver functions of rats**

Biomarker	Control	20 Hz	<i>P</i> *	<i>d</i> **
Glucose (mmol/l)	8.005 ± 1.72	7.54 ± 0.56	0.6255	0.31
CK (UI/h)	617.2 ± 196.2	694.3 ± 182.2	0.5861	5.56
Albumin (g/dl)	3.3 ± 0.2	3.5 ± 0.3	0.1035	1.03
Alkaline phosphatase (U/ml)	120.1 ± 20.1	135.2 ± 40.5	0.5290	0.82
TGP (UI/I)	58.8 ± 21.04	72.5 ± 14.84	0.3282	0.11
TGO (UI/I)	124.8 ± 45.9	161.3 ± 15.94	0.1837	0.51
γGT (UI/I)	6.7 ± 2.5	2.2 ± 0.8	0.0140	1.80

*P*\* represents a statistical parameter related to the ANOVA test.

*d*\*\* represents a statistical parameter related to the size of the samples.

**Table 2. Effects of 20 Hz vibration on plasma biomarkers related to the lipid metabolism in rats**

Biomarker	Control	20 Hz	<i>P</i> *	<i>d</i> **
Total cholesterol (mg/dl)	43.16 ± 11.30	42.00 ± 14.89	0.9053	0.10
Triglycerides (mg/dl)	21.33 ± 13.96	14.00 ± 5.09	0.3619	0.53
HDL (mg/dl)	40.83 ± 9.98	34.00 ± 8.0	0.3266	0.03
LDL (mg/dl)	1.50 ± 0.98	2.40 ± 1.27	0.3047	0.10
VLDL (mg/dl)	4.46 ± 1.15	2.80 ± 0.98	0.0470	1.60

*P*\* represents a statistical parameter related to the ANOVA test.

*d*\*\* represents a statistical parameter related to the size of the samples.

**Table 3. Effects of 20 Hz vibration on the biochemical values of biomarkers related to kidney and pancreas functions**

Biomarker	Control	20 Hz	<i>P</i> *	<i>d</i> **
Lipase (μg/ml)	4.95 ± 0.75	5.85 ± 0.70	0.1299	1.20
Amylase (μg/l)	2360.16 ± 265.21	2959.75 ± 559.61	0.1009	2.26
Urea (mg/ml)	54.83 ± 9.02	62.00 ± 14.30	0.4289	0.79
Creatinine (mg/ml)	0.36 ± 0.08	0.47 ± 0.15	0.2432	1.38

*P*\* represents a statistical parameter related to the ANOVA test.

*d*\*\* represents a statistical parameter related to the size of the samples.

**Table 4. Effects of 20 Hz vibration on the number of white blood cells and platelets in the blood of rats**

Cell type	Control	20 Hz	<i>P</i> *	<i>d</i> **
WBC (10 <sup>9</sup> /L)	4353 ± 359	6040 ± 879	0.0120	0.33
Neutrophils (%)	2.60 ± 0.91	3.31 ± 2.21	0.5741	0.78
Lymphocytes (%)	5.002 ± 2.38	2.59 ± 1.38	0.1301	0.68
Monocytes (%)	0.032 ± 0.014	0.037 ± 0.018	0.6764	0.36
Eosinophils (%)	0.037 ± 0.019	0.018 ± 0.006	0.1051	1.75
Basophils (%)	0.071 ± 0.042	0.053 ± 0.027	0.4980	1.00
Platelets (10 <sup>3</sup> cells/μl)	922 ± 152	1137 ± 105	0.5888	1.41

*P*\* represents a statistical parameter related to the ANOVA test.

*d*\*\* represents a statistical parameter related to the size of the samples.

some biomarkers in the blood of rats exposed to the vibration for 1 min/day for one week. The following discussion focuses on the effects of this vibration on the metabolism, which has also influenced the selection of the biomarkers.

$\gamma$ GT is an enzyme related to liver functions (34). As such, the significant ( $P < 0.05$ ) decreases in the  $\gamma$ GT concentration found in our study suggest that 20 Hz vibration may be effective in producing an effect associated with liver functions. In an investigation on men, Pettersson *et al.* (34) described that moderate physical exercise resulted in transient elevations of liver functional tests although these authors did not find changes in the concentration of  $\gamma$ GT. They did, however, find an increase in CK levels, another enzyme related to liver functions (34). Considering our findings (Table 1) with rats submitted to a WBV, that can elicit vigorous muscle contractions (17), no alterations in CK, TGP, TGO and alkaline phosphatase concentrations were found. Gojanovic *et al.* (17) found that five participants (25%) who took part in a training program also showed a significant increase in post-exercise CK levels (>double baseline concentrations). In studies with rats, Naghii *et al.* (30) reported that exposure to vibrations with frequencies of 10-50 Hz for eight weeks resulted in significant differences in plasma levels of CK, and that these plasma CK levels were significantly higher in the vibration group compared to the controls. Although the current study (Table 1) did not show a significant change in serum CK levels, there was a slight increase (not significant) and it could be postulated that exposure to other frequencies for a longer period of time may be effective in controlling these concentrations following exercise. This consideration is in agreement with the findings reported by Naghii *et al.* (30). It is, therefore, hypothesized that lower frequencies (in this case 20 Hz for one week) can assist in maintaining, at least in part, the integrity of the functions of the organs or tissues related to CK, as also reported by other authors (2).

Although some authors have reported that WBV exercises could reduce glucose levels in patients with diabetes mellitus type 2 (DM-2) (3), our findings did not show a change in the concentration of this biomarker in the blood of rats. This may be because this study was conducted in healthy animals. Di Loreto *et al.* (14) reported in their study that vibration at 30 Hz resulted in a slight reduction (not significant) in blood glucose levels in patients with DM-2. Another study by Behboudi *et al.* (3) found a significant decrease in fasting glucose after WBV-exercise compared to a control group of patients with diabetes. These findings suggest that for diabetes patients, exposure to vibration at a higher frequency may be beneficial. However, whether this effect can be attributed

to vibration or exercise still needs to be tested empirically.

The effects of 20 Hz vibration on the levels of some biomarkers of lipid metabolism in the blood also varied. The concentration of VLDL decreased significantly ( $P < 0.05$ ) and the concentration of the triglycerides, HDL and LDL, were altered. Our findings were similar to those reported by Naghii *et al.* (30) who determined that the plasma lipid concentrations (cholesterol and triglycerides) in rats submitted to vibrations of 10-50 Hz for eight weeks did not change. It is possible to speculate that this result could be associated with specific characteristics of the metabolic pathways associated with lipid metabolism which could be not influenced by the vibrations. Goto and Takamatsu (18) found an increase in serum fatty acids in healthy individuals subjected to vibration by exercising on a vibrating platform, a finding which might be important for further research in the obese population.

No significant differences were found regarding the urea and creatinine for renal functions, lipase and amylase concentrations following exposure to 20 Hz vibration (Table 3). In agreement with our data, Naghii and Hedayati (31) did not observe alteration due to WBV in the concentration of another biomarker uric acid associated with the renal function. Nevertheless, Pereira *et al.* (33) demonstrated an uptake of a  $^{99m}\text{Tc}$ -radiopharmaceutical in the kidney and bladder of rats subjected to vibration suggesting that this effect was not associated with the physiology of the kidneys. This suggests that either WBV (20 Hz, 1 min) would not be safe and, therefore, indicated that in persons with kidney or bladder conditions, side effects should be closely monitored.

In the blood, after exposure at 20 Hz vibration for one week, the number of leukocytes increased significantly (Table 4). The exposure had little effect on the concentrations of neutrophils, monocytes, basophils, platelets and lymphocytes, and these changes were not significant. Although this study did not measure bone mineral density, the decrease of the number of lymphocytes, although not significant, does agree with the findings reported by Tossige-Gomes *et al.* (45) in which the proliferative response of  $\text{TCD4}^+$  cells to vibration showed a significant decrease in the WBV group compared to the control group. Tossige-Gomes *et al.* (45) also suggested that the addition of WBV exercise might modulate the immune process mediated by the T-cell (23). In general, our findings (Table 4) are in agreement with Pawlak *et al.* (32) that submitted Wistar rats to vibrations of 50 Hz for three and six months, and they also observed no significant alteration in lymphocytes, monocytes, granulocytes, hemoglobin, and hematocrit, as well as interleukin-1b, interleukin-10,

interleukin-6, and vascular endothelial growth factor levels in comparison to control group, although these authors did not also find alteration in the number of white blood cells. As the frequencies used in our investigations (20 Hz) and in the study of Pawlak *et al.* (32) were different, further investigation into optimal frequencies is warranted. This notion is also supported by Garman *et al.* (16) who suggested that the enhancements in BFR/BS and MS/BS reported in his study were apparently acceleration-dependent.

A limitation of this investigation is related to the absence of a baseline to the values of the concentrations of the biomarkers due to the fact that the rats used were a homogenous group, bred and exposed to the same environmental and stress conditions. Furthermore, as the animals were not in the same place in each side of the teeterboard, there was a small difference in the peak-to-peak displacements, that were 1.5 and 3.8 mm considering the center line of the rats, that the animals were exposed to. According to Rauch *et al.* (38), these peak-to-peak displacements generate peak acceleration of 11.83 and 29.94 ms<sup>-2</sup>, respectively. Moreover, the overall acceleration is not greatly different to impact on the effect of the vibration on blood biomarkers.

As reported by several authors, the action mechanism associated with the effects of the vibrations generated in oscillating/vibratory is highly complex and seem to be associated with several parameters, and the frequency of the vibration would be important in generating biological effects (9, 36, 39, 40). A comparison of results presented in this study with the findings reported by other authors has revealed what is necessary in further investigations. It would be important to consider in the studies various controlled conditions to try to better understand the physiological mechanisms and to establish a relationship with several parameters, including frequency, length of the study and peak-to-peak displacement. It is noteworthy that the protocols used in the investigations with animals were different (11, 16, 30, 32, 33, 41).

In conclusion, important results were reported on the effects of 20 Hz vibration generated by an oscillating platform on biochemical blood markers in Wistar rats. While these findings do not explain these mechanisms, the reported data may permit speculations on changes that occur following exposure to a frequency of 20 Hz. These data should be helpful in designing further studies aimed at better understanding of mechanisms and for optimizing dosage parameters.

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