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Full Length Research Paper

Effect on osmotic fragility of red blood cells of whole blood submitted to vibrations in an oscillating platform

Milena O. B. Monteiro[1,2], Nelson de Souza Pinto[1,3], Pedro J. Marin[4,5], Sebastião David Santos-Filho[1,6]*, Mario Bernardo-Filho[1,7]

[1]Departamento de Biofísica e Biometria, Instituto Roberto Alcantara Gomes, Universidade do Estado do Rio de Janeiro, Rio de Janeiro, RJ, Brasil.
[2]Programa de Pós-Graduação em Ciências Médicas, Universidade do Estado do Rio de Janeiro, Rio de Janeiro, RJ, Brasil.
[3]Programa de Pós-Graduação em Ciências da Saúde, Universidade Federal do Rio Grande do Norte, Natal, RN, Brasil.
[4]Laboratory of Physiology, European University Miguel de Cervantes, Valladolid, Spain.
[5]Research Center on Physical Disability, ASPAYM, Castilla y León, Spain.
[6]Centro de Ciências da Saúde, Universidade Severino Sombra, Vassouras, RJ, Brasil.
[7]Coordenadoria de pesquisa, Instituto Nacional do Câncer, INCa, Rio de Janeiro, Brasil.

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Whole body vibration (WBV) exercises in oscillating platforms (OP) have emerged in sports and in the rehabilitation procedures of clinical disorders. The aim of this work was to verify the effects of vibrations on the osmotic fragility (OF) of red blood cells (RBC) isolated from whole blood submitted to OP. Heparinized blood samples were withdrawn from German dog, and distributed in tubes that were divided in three sets. A set of tubes was submitted to 0 Hz (control), the second set of tubes was submitted to 10 Hz and the third set to 20 Hz for 1 min in an OP. Then the OF of the RBC was determined, and the results indicate that the vibration promotes an increase on the hemolysis from 9 ± 1 to 20 ± 2% with 10 and 20 Hz, respectively. The shape of the OF curves for 10 and 20 Hz were similar. A significant difference ($P<0.05$) was found when a comparison was done with the control curve (0 Hz) and the experimental sets (10 and 20 Hz). Our findings therefore indicate that the increase of the frequency from 10 to 20 Hz seems to induce damage on the RBC membrane and this effect was dependent on the frequency. In addition, we suggest precaution with the use of the vibration generated in OP.

Key words: Vibratory platform, hemolysis, frequency, erythrocyte membrane, blood.

INTRODUCTION

Vibration is a mechanical stimulus. It is a periodic oscillation with alteration of force, acceleration and displacement over time (Rittweger, 2010). Considering the clinical application of the vibration, vibration exercise in a physical sense, is a forced oscillation in which the energy that is generated in a system is transferred from this generator of the vibrations to a receptor (human being) in the whole body or to a part of it (Cardinale and Wakeling, 2005; Rittweger, 2010). The use of the vibrations, as whole-body vibration (WBV) exercises on oscillating platform (OP) have emerged in sports training and in the rehabilitation procedures of various clinical disorders. Zepetnek et al. (2009) cited five factors that are related to the response of the human skeletal system to the WBV exercises, as vibration direction (vertical or oscillatory altering), vibration frequency [measured in hertz (Hz)], vibration magnitude measured as amplitude [displacement of the vibration in millimeters (mm)] and acceleration [gravitational units (m/s^2) or (g)], duration of the work in the OP and the body position/posture on the platform.

Rittweger (2010) reported that, although, vibration exercise nowadays is being broadly available to exerci-

*Corresponding author. E-mail: sdavidsfilho@gmail.com. Tel/Fax: 55-21-2868-8332.

sers and patients, it seems that this exercise modality is still largely unknown to the scientific community. Prisby et al. (2008) discussed that there is a paucity of data in the literature regarding the physiological modulation of WBV on other organ systems and tissues. Vibrations can be also found in some vibratory tools, such as impact wrenches and chipping hammers. The workers using percussion vibratory tools are often strongly exposed to impulse vibration directly on their hands and palms (Pelmear and Wills, 1997). An association between hand–arm vibration syndrome and exposure to impulse vibration is now recognized for workers who occupationally use handheld power tools (Pelmear et al, 1995).

The number of publications involving whole body vibrations cited in the PubMed is still limited when compared with other physical agents used in health science. Some authors have reported important biological effects associated with the action of the vibrations (Prisby et al., 2008; Ando et al., 2005). Ando et al. (2005) reported that the fragmentation of red blood cells (RBC) can be seen when they are exposed to excessive mechanical stress in circulation. Moreover, Ando et al. (2005) discussed that hemolysis occurs when a part of the body is exposed to intense impulses repeatedly in such activities as marathons (Buckle, 1965), karate (Streeton, 1967), and conga drum playing (Kaden, 1970). Davidson (1964) also suggested that the hemolysis takes place through the pressure applied from outside on blood vessels when hands and feet are hit directly on solid objects. More also, Ando et al. (2005) evaluated the damage induced in red blood cells by exposure to impulse vibration generated in an artisanal device, and each vibration caused damage to these blood cells. It was observed that the higher the peak acceleration and the longer the exposure duration, the more the damage to the red blood cells.

Moreover, Ando et al. (2005) also reported that no studies have been conducted concerning the pathological effects, such as hemolysis vibrations on the circulatory system. Hence, combining this information, the aim of this work was to verify the effects of vibrations on the osmotic fragility (OF) of RBC isolated from whole blood submitted to OP.

MATERIALS AND METHODS

Ethical approval

The procedures with the animal in our experiments were approved by the Ethical Committee of the Instituto de Biologia Roberto Alcantara Gomes, Universidade do Estado do Rio de Janeiro with the number CEA/024/2009. A professional (Veterinary Physician) was always present in the various steps of the manipulations of the animal.

Blood samples

Blood was withdrawn from a male dog (*Canis familiaris*) with 60 ± 5 g of weight. Blood samples of 50 ml were withdrawn by venous access (Brachial vein) in sterile syringe (60 ml) and with anticoagulant (2 ml of heparin); this syringe was coupled to a scalp 19. This volume was about 0.8% of the total blood volume of the animal. The blood (50 ml) was used in 24 isolated experiments. The blood was withdrawn by a veterinarian responsible for the animal. After that, the dog was maintained in a special room with controlled temperature and food and water *ad libitum* up to its total recovery. The animal was manipulated again after 15 days to obtain another sample of blood (50 ml) in the repetition of the experiment. This procedure was important to prevent infections and to distress the animal. The two samples of blood (50 ml) enabled the 48 experiments that were used in the statistical analysis.

Characteristics of the oscillating platform

The platform used in the experiment is an oscillating system (Novaplate fitness evolution, DAF, Produtos Hospitalares Ltda, São Paulo) with reciprocating vertical displacements on the left and right side of a fulcrum. It is a side-alternating vibration device working as a teeterboard (28 cm × 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.

Experimental procedure

In each experimental study, three sets of tubes were used. A set of tubes (eight) was submitted to 0 Hz (control), the second set of tubes (eight) was submitted to 10 Hz and the third set (eight) to 20 Hz in an oscillating platform. Heparinized blood samples (1.5 ml) were distributed in assay tubes (2.0 cm in size and 0.5 cm in diameter). The tubes were under the platform of the oscillating platform and fixed with adhesive tape, 17 cm from the centre of the teeterboard. The amplitude of the vibration in this distance from the centre was 4.14 mm. Photos of the tubes in the platform were acquired with a camera (E850, 8 megapixels, General Electric Imaging Co., Torrance, USA). After the fixation of the tubes, the frequency was selected (10 or 20 Hz) and the time (1 min) that were utilized in the assay. This procedure was repeated for each experimental set. In the control set, the tubes with blood were not submitted to vibrations in the platform. In Figure 1 are shown the photos with the position of the tubes in the platform of the oscillating platform. Figure 1a shows an overview of the tubes in the platform, Figure 1b shows the tubes on the left of the teeterboard and Figure 1c shows the tubes on the right of the teeterboard.

Osmotic fragility

After the experiment in the oscillating platform, the blood samples were centrifuged (3000 rpm, 5 min, clinical centrifuge). A volume of 1 ml of the supernatant was separated to verify the absorbance at 540 nm in a spectrophotometer (Analyser 800M, Optromic Instrumentos Científicos Ltda, Rio de Janeiro, Brazil) using 0.9% NaCl as a blank. The hemolysis was evaluated by a factor of comparison of osmotic fragility. This factor was determined dividing the absorbance of the supernatant of treated sample of blood (10 and 20 Hz) by the supernatant of the control sample (0Hz). The blood was homogenized and 0.06 ml was used for counter tube prepared with different hypotonic NaCl (0.02 to 0.12 M). It was homogenized three times gently in each tube, and centrifuged again (3000 rpm, 5 min, clinical centrifuge). Finally, 1 ml of the supernatant was then utilized to obtain absorbance at 540 nm; used for each sequence in the tube with 0.12 M NaCl as blank.

Statistical analysis

The data of mean of hemolysis percentage in each interval in the

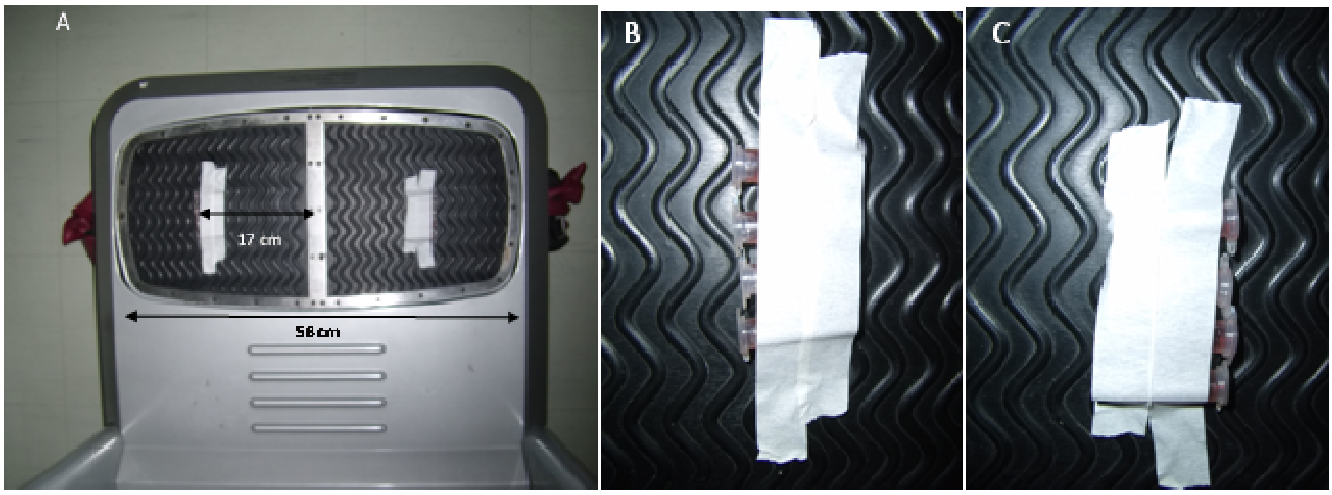

Figure 1. A, An overview of the tubes on the teeterboard of the platform. The tubes were 17cm from the centre of the platform; B, view of the tubes on the left side of the teeterboard of the platform; C, view of the tubes on the right side of the teeterboard of the platform.

fragility curve were presented as means ± standard deviation. Paired t-test was used to compare each one of the intervals; I, II and III (Cavalcanti et al., 2003) between control and each one of the treated isolated sets (control and 10 Hz, and control and 20 Hz). A significance level at $p < 0.05$ was adopted. InStat Graphpad software was used to perform statistical analysis (GraphPad InStat version 3.01 for Windows 95/NT, GraphPad Software, San Diego, USA).

RESULTS AND DISCUSSION

Figure 2 shows the comparison between the factor of osmotic fragility of the control (sample of blood not submitted to vibration) and the effect of the frequencies used in the experiment. The increase of the frequency of the vibration caused an increase of the osmotic fragility. The vibration promoted an increase on the hemolysis from 9 ± 1 to 20 ± 2% with 10 and 20 Hz, respectively. Figure 3 shows the effect of the vibration in the oscillating platform on the whole blood submitted to a frequency of 10 and 20 Hz, respectively. We can observe that the effect of 10 Hz was similar to that of 20 Hz. In both evaluations, an increase in the osmotic fragility of the RBC was found. Figure 4 shows the statistic analysis of the intervals of the NaCl concentrations proposed by Cavalcanti et al. (2003) in the osmotic fragility curve. It shows a significant ($p<0.05$) hemolysis in interval II in both frequencies utilized.

Different experimental models (Prisby et al., 2008; Rittweger, 2010) have been used to evaluate the biological effects of vibration produced in an oscillating platform. Our findings obtained with isolated whole blood submitted to vibration reveal an increase of the osmotic fragility with the used frequencies. The importance of the investigations about the effect of vibrations generated in OP is due to the possible several clinical applications of them (Cardinale and Wakeling, 2005; Pinto et al, 2010a; Pinto et al, 2010b; Rittweger, 2010; Pinto et al, 2011). In addition, the number of publications found in the PubMed using the keyword “whole body vibration” was 766 (August 19th, 2011), while in another physical agents, “laser”, it was 184061, or “infrared” it was 81508. Moreover, no publications were found with “whole body vibration” and “red blood cells”. A search using the keywords “impulse vibration” and “red blood cells” revealed one publication (Ando et al., 2005). Rittweger (2010) reported that more research is needed in order to better understand the specific therapeutic potential of vibration as an exercise model. Moreover, there seems to be a certain need for studies to assess any potential long term risks.

Ando et al. (2005) showed that vibration was also capable to cause damage to red blood cells *in vitro*. The peripheral blood smears clearly showed some fragmentations of red blood cells after exposure to impulse vibration. The damage seems to depend on the combined effect of peak acceleration and exposure duration. The greater and the longer the exposure to impulse vibration, the more damage caused to red blood cells. This finding suggests an effect on red blood cells related to the peak acceleration and exposure duration of impulse vibration.

The main value of the osmotic fragility test, as commonly used in clinical practice, is to confirm important morphological abnormalities of a blood sample, such as the presence of leptocytes and spherocytes (Kumar et al., 2010). However, the osmotic fragility of red cells not only reflects the peculiarities in average membrane and cytoplasm properties of a given sample, but it can also provide information about the distribution of those properties within the sample itself. Several authors (Warang et al., 2011; Rocha et al., 2011; Chaibunruang

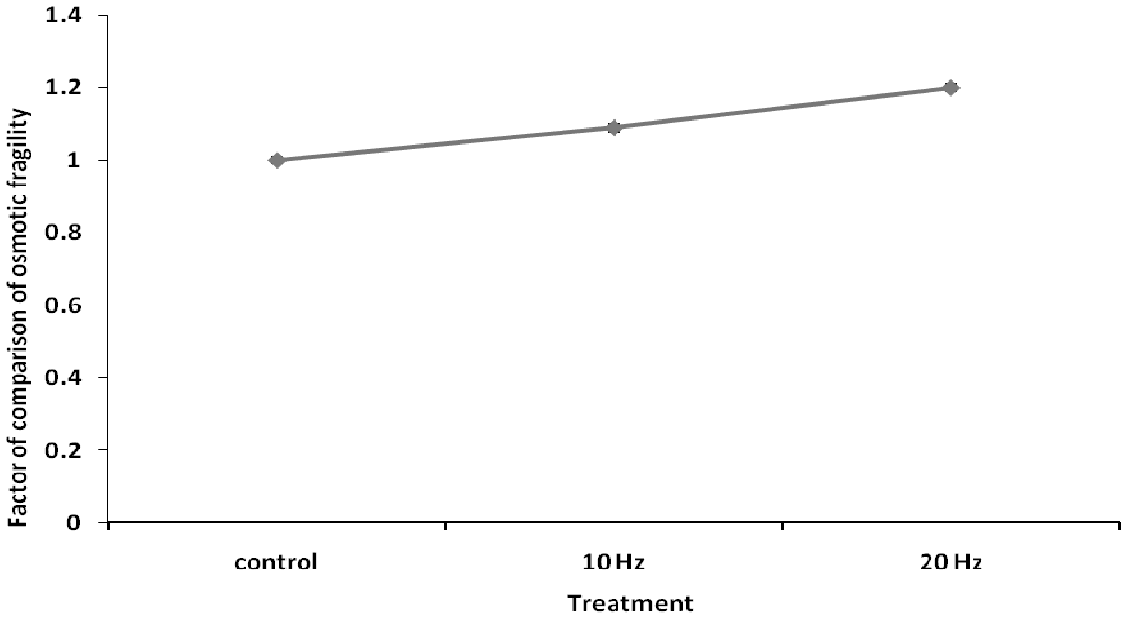

Figure 2. Factor of comparison of osmotic fragility. After the experiment in the vibratory platform, the blood samples were centrifuged. A volume of 1 ml of the supernatant was separated to verify the absorbance at 540 nm in a spectrophotometer using NaCl 0.9% as a blank. A factor of comparison of osmotic fragility was made dividing the absorbance of the supernatant of the treated samples (10 and 20 Hz) by the supernatant of control sample of blood.

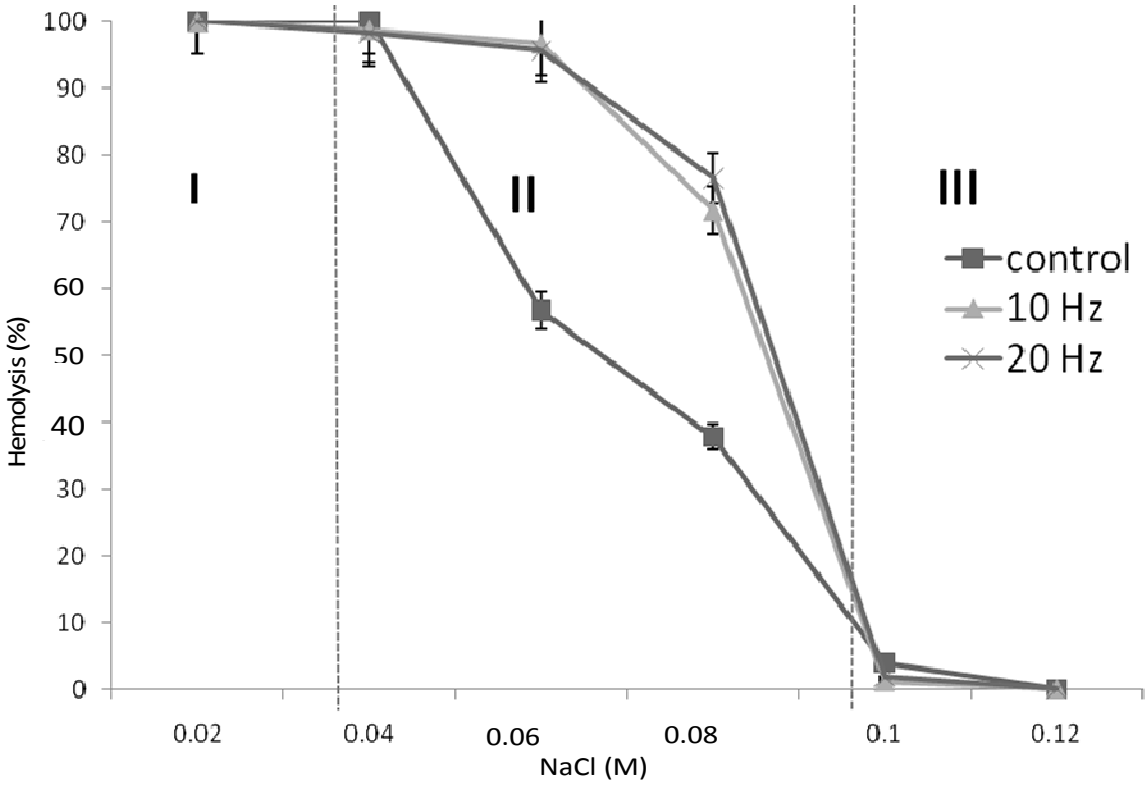

Figure 3. Fragility osmotic curve of whole blood. The blood was homogenized and samples (0.06 ml) were transferred to tubes with different hypotonic NaCl (0.02 to 0.12M). They were gently homogenized three times and centrifuged. It utilized 1 ml of the supernatant to obtain absorbance at 540 nm, using the tube with NaCl 0.12 M as blank. I, II and III indicate the intervals used for do the statistical analysis.

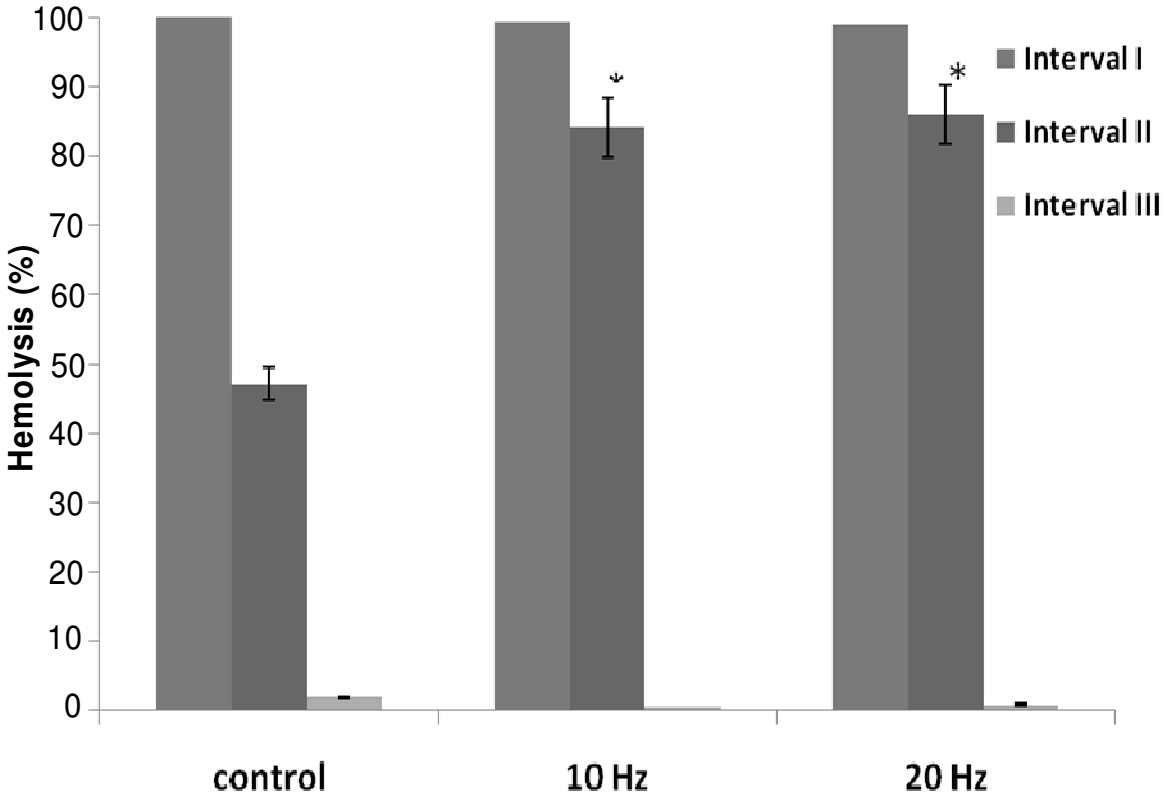

Figure 4. Statistical analysis. The blood was homogenized and 0.06 ml was used for counter tube prepared with different hypotonic NaCl (0.02 to 0.12 M). It was homogenized three times gently in each tube, and centrifuged again at 3.000 rpm/ 5 min. Finally, we utilized 1 ml of the supernatant to obtain a absorbance at 540 nm, used for each sequence tube with NaCl 0.12 M as blank. The curve was divided in three intervals, I (0.02 to 0.04 M), II (0.06 to 0.08 M) and III (0.10 to 0.12 M). A statistical analysis was done; *p<0.05.

et al., 2010) have been able to correlate differences in cell density and osmotic fragility with the age of the red blood cells in terms of "young," "mature," and "old" cells. A more systematic and quantitative classification of normal and pathological red blood cells may be obtained by developing a model that relates the osmotic properties of a given blood cell sample with the morphological characteristics of its distribution. When the external osmotic pressure is reduced arbitrarily, the cell volume increases according to a relationship which deviates, to a greater or lesser extent, from ideal behavior. In particular, at the onset of hemolysis, a distribution of critical volumes associated with the distribution of critical osmotic pressures can be expected to hold (Lewis et al., 2006).

Our findings therefore show that when vibration frequency is increased from 10 to 20 Hz, the osmotic fragility of RBC can be increased, as demonstrated by the hemolysis (Figure 2). Moreover, it indicates that the increase of the frequency from 10 to 20 Hz seems to induce damage on the RBC membrane and this effect was dependent on the frequency (Figures 2 and 3). These results are in agreement with Ando et al. (2005), although the source used by these authors to generate vibration was different.

Conclusion

Vibration is therefore capable of altering the osmotic fragility of the RBC probably due to damages on the erythrocyte membrane, and this effect was dependent on the frequency. Moreover, our findings could aid a better understanding of the effect of the whole body vibration exercise on humans. Furthermore it is relevant to consider that our results were obtained with blood isolated from a dog, hence, we suggest precaution with the use of the vibration generated in oscillating platform.

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