Effect Of Resistance Training On Myocardial Contractility In Vitro After Sleep Deprivation

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Introduction

Sleep duration is one of the determining factors of cardiovascular health. Studies have demonstrated that a shorter time allocated to sleep is closely associated with increased prevalence and incidence of cardiovascular diseases, such as hypertension. Among the possible mechanisms, an increased sympathetic activity and catabolism is evidenced by increased levels of catecholamine and cortisol/corticosterone which leads to molecular and morphological changes that may negatively affect cardiac tissue.

In this scenario, resistance training stands out as a possible tool to minimize or even reverse the impact of sleep debt on cardiovascular health. In addition to reducing resting arterial pressure, left ventricular systolic pressure and heart rate, resistance training can also increase papillary muscle isometric strength and its contractile performance. Also, due to its effect on endocrine axes, resistance training can mitigate the catabolic effect of sleep deprivation.

Despite studies showing the impact of sleep debt on cardiovascular health, little is known about its effects...
on cardiac contractile machinery and the role of resistance training as a preventive tool. Therefore, the aim of this study was to evaluate the effect of high-intensity resistance training on myocardial contractility in rats subsequently subjected to paradoxical sleep deprivation.

**Methods**

Using a convenience sample, forty 3-month old male Wistar rats, weighing 300-350 g, obtained from the Center for the Development of Experimental Models in Medicine and Biology of Sao Paulo Federal University was used in the experiment. The animals were kept in polypropylene boxes in a temperature-controlled room (22 ± 1ºC), light/dark cycle (12:12h), and given water and food *ad libitum*.

All procedures were performed according to the Guide for the Care and Use of Laboratory Animals - National Research Council, NIH Publication N° 85-23 (revised 2011). The study protocol was approved by the Ethics Committee (#0764/10).

The animals were distributed into four groups: control (CTRL, n = 10), resistance training (REST, n = 10), paradoxical sleep deprivation (PSD) for 96 hours (PSD96, n = 10), and resistance training followed by 96-hour PSD (REST/PSD96, n = 10). During the experiment, animals of the CTRL group were kept in the boxes and manipulated exclusively for laboratory routine practices.

**Resistance training**

Resistance training was performed for 8 weeks as described elsewhere. A ladder (110 cm high, 18 cm wide, 2 cm space between steps) was placed at an 80º inclination, and a resting chamber (20x20x20 cm) was placed on the top of the ladder. The animals climbed the ladder carrying weights attached to the base of the tail, and the weights were gradually increased with exercise progression.

Familiarization of the animals with the exercise apparatus was performed during three consecutive days by nine repetitions per day. After this period, the maximum load (ML) test was performed, which was repeated every week.

Training sessions consisted of 4-8 sets of climbing, using progressive loads, interspaced with 60-second intervals. The animals had to perform 8 - 12 repetitions to go from the base to the top of the ladder. The load was progressively increased from 50% of maximum load in the first series, through 75%, 90% until 100% of maximum load in the fourth series. After that, resistance was increased in increments of 30 g per attempt until failure. Training was performed in the afternoon, 5 times a week, from Monday to Friday. Monday session was replaced by the maximum load test. In order to prevent overtraining caused by the high intensity of exercise, a prophylactic rest was adopted at weeks 6, 7 and 8; in these weeks, the animals trained on Monday, Tuesday, Thursday and Friday. Forty-eight hours after the last training session, the CTRL and REST animals were euthanized and the PSD96 and REST/PSD96 groups underwent the PSD protocol.

**Paradoxical sleep deprivation**

PSD was conducted for 96 hours by the modified multiple platform method, which totally promotes the total suppression of paradoxical sleep and the decrease in slow wave sleep by 37%. Five socially stable animals were placed inside a stainless steel tank (123 cm length x 44 cm height x 44 cm width) on round platforms 10 cm distant from each other. The tank was filled with room temperature water until 1 cm below the platform surfaces, so that the animals could walk from one platform to another one. When paradoxical sleep was achieved, the animals woke up as they touched the water due to muscle atonia accompanying this sleep stage. During the protocol, the room was maintained at optimal conditions, i.e., 22±1°C, light-dark cycle (12h) and water and chow *ad libitum*. The CTRL group was kept in the same room to experience the same conditions.

**Isolated cardiac muscle mechanics**

Immediately after the PSD protocol for PSD96 and REST/PSD96 groups, or 48 hours after the last resistance training sessions for the REST group, or after a corresponding period for the CTRL group, *in vitro* preparations were made of papillary muscle isolated from the left ventricle.

Rats were anesthetized with urethane (Urethanechloride, 0.3 ml/100 g, *i.p.*, Sigma-Aldrich, St. Louis, Mo, USA), and the heart was rapidly removed and placed in Krebs-Henseleit solution previously bubbled with oxygen 100% at 30°C. Papillary muscle was dissected from the left ventricle and its tendinous and parietal ends were attached to a strength transducer by stainless steel rings. The papillary muscle was then suspended vertically in a glass chamber containing Krebs-Henseleit (mM) solution.
(132 NaCl, 4.69 KCl, 1.5 CaCl, 1.16 MgSO₄, 1.18 KH₂PO₄, 5.50 glucose and 20 HEPES), buffered at pH 7.4. The ring attached to the lower end of muscle was connected to the hook fixed to the glass chamber, and the upper ring was connected to an isometric force transducer (model FT03E, Grass Instrument, Quincy, MA) via a stainless steel wire. The force transducer was attached to a micromanipulator for muscle length adjustments. The papillary muscle was electrically stimulated by two parallel platinum electrodes using square wave pulses of 5ms of duration, frequency of 0.2Hz, and voltage approximately 20% higher than the minimum required to induce a maximal muscle response. After 60 minutes of stabilization of the preparation in isotonic condition and low preload (0.4g), the muscle was loaded to contract isometrically and then stretched until the peak of the length-tension curve was reached (Lmax; diastolic length of the muscle associated with maximal isometric tension). The tests were performed in Lmax and isometric tension was assessed by force normalized for muscle fiber cross-sectional area (CSA) (g. mm⁻²). Muscle CSA was calculated from molecular weight and length of the fibers, based on the assumption that the muscle was a uniform cylinder of specific gravity 1.04.

The following outcome measures were assessed for contraction function: developed tension (DT/ g.mm⁻²), resting tension (RT g.mm⁻²), temporal variation of contractile force (+dT/dt; g.mm⁻².s⁻¹), temporal variation of relaxation force (-dT/dt; g.mm⁻².s⁻¹), time to DT peak (TTP; ms) and time to a 50% decrease in maximum DT (RT50% ms). Mechanical behavior of papillary muscles was assessed (1) at baseline; (2) for length-tension relationship (variation of muscle length as a function of 92%-100% Lmax); (3) for post-rest potentiation (after 10, 20, 30, 60 and 120 seconds of rest); and (4) for contractile response to calcium (Ca²⁺) (increase in Ca²⁺ concentration from 1.5mM to 2.5mM in the bathing solution).

Statistical analysis

Statistical analysis was performed using the Statistica® software (Stat Soft, Inc, version 12.0). Measures of central tendency were calculated for descriptive analysis of data. Data normality was examined by the Shapiro Wilk test. Data with parametric distribution were analyzed by one-way or two-way ANOVA with Duncan post-hoc test, and after Z-score transformation, non-parametric data were analyzed by ANOVA. In the analyses of length-tension relationship and post-rest potentiation, linear regression was used for comparisons between slopes and between areas under the curves, respectively. Values in each group (slope and area under the curve) were analyzed by ANOVA. Statistical significance was set at α = 0.05.

Results

Biometric parameters of papillary muscle

No difference in papillary muscle mass was found between the groups (F(3,23) = 0.16005, p > 0.05). There was a significant decrease in Lmax in the PSD96 (p = 0.03) and REST/PSD96 (p = 0.002) groups as compared with CTRL, and Lmax was significantly different between REST/PSD96 and PSD96 (p = 0.005), (F(3,23) = 6.0487, p < 0.01). In addition, CSA significantly increased in the REST (p = 0.02), PSD96 (p = 0.02) and REST/PSD96 (p = 0.01) groups compared with CTRL (F(3,19) = 3.1706, p < 0.05) (Table 1).

Contractile mechanics of papillary muscle under isometric contraction (baseline)

There was a significant decrease in DT in the REST/PSD96 group as compared with the CTRL group (F(3,23) = 33049, p = 0.009). RT was lower in PSD96 than in REST (F(3,22) = 3.6623, p =0.01). A significant decrease in +dT/dt was observed in the PSD96 (0.006) and REST/PSD96 groups as compared with CTRL (F(3,20) = 8.0313, p < 0.05), whereas -dT/dt significantly decreased in PSD96 (F(3,26) = 2.7244, p = 0.01) (Figure 1).

Similar behavior of time parameters of cardiac contraction was observed between the groups (F(3,23) = 0.00, p > 0.05). However, with respect to RT50%, a prolongation of muscle relaxation was observed in PSD96 as compared with the other groups (F(3,26) = 3.9344, p < 0.05).

Contractile mechanics of papillary muscle in length-tension relationship

Slopes of DT were significantly lower in the REST (p = 0.02), PSD96 (p = 0.03) and REST/PSD96 (p = 0.0001) groups than in CTRL group, and in the REST/PSD96 when compared with the REST (0.03) and PSD96 (p = 0.02) groups (F(3,24) = 7.0880, p < 0.01). In addition, a reduction in RT was observed in PSD96 as compared with REST (F(3,29) = 2.5483, p < 0.05) (Figure 2).

Slopes of +dT/dt in PSD96 and REST/PSD96 were significantly lower than in CTRL (p < 0.01) and REST (p < 0.05), and significantly higher in PSD96 than...
Table 1 – Effect of resistance training and paradoxical sleep deprivation on biometrical parameters of papillary muscle

<table>
<thead>
<tr>
<th>Variables</th>
<th>CTRL</th>
<th>REST</th>
<th>PSD96</th>
<th>REST/PSD96</th>
</tr>
</thead>
<tbody>
<tr>
<td>Papillary muscle mass (mg)</td>
<td>5.96 ± 2.02</td>
<td>5.98 ± 1.43</td>
<td>6.35 ± 0.89</td>
<td>5.77 ± 1.64</td>
</tr>
<tr>
<td>Lmax (mm)</td>
<td>5.86 ± 0.84</td>
<td>5.71 ± 0.73</td>
<td>4.91 ± 0.64</td>
<td>4.40 ± 0.60</td>
</tr>
<tr>
<td>CSA (mm²)</td>
<td>1.00 ± 0.27</td>
<td>1.30 ± 0.19</td>
<td>1.29 ± 0.12</td>
<td>1.35 ± 0.21</td>
</tr>
</tbody>
</table>

Data in mean ± standard deviation; Lmax – maximum papillary muscle length; CTRL: control group; REST: resistance training group; PSD96: paradoxical sleep deprivation for 96 hours; REST/PSD96: resistance training followed by 96-hour paradoxical sleep deprivation; CSA: cross-sectional area; *different from CTRL; † different from REST group

Figure 1 – Effect of resistance training and paradoxical sleep deprivation on contractile mechanics of the papillary muscle under isometric contraction. One-way ANOVA with Duncan post-hoc test. Data expressed in mean ± standard error, with significance set at p = 0.05. DT: maximum developed tension; RT: isometric resting tension; +dT/dt: temporal variation of contraction force; −dT/dt: temporal variation of relaxation force; TTP: time to developed tension peak; RT50%: time required for maximum developed tension to decrease by 50%; CTRL: control group; REST: resistance training group; PSD96: paradoxical sleep deprivation for 96 hours; REST/PSD96: resistance training followed by 96-hour paradoxical sleep deprivation. *significantly different from CTRL; † different from REST group; ‡ different from all groups
in REST/PSD96 (p = 0.01), (F(3,21) = 15.741, p < 0.01). No difference in -dT/dt slopes was found between the groups (F(3,20) = 2.4210, p > 0.05).

Also, TTP was not different between the groups. The PSD96 group had higher RT50% for all stretching percentages tested as compared with the other groups (F(3,25) = 5.1010, p < 0.05) (Figure 3).

**Contractile mechanics of papillary muscle in post-rest potentiation**

There was a decrease in DT in REST/PSD96 compared with CTRL (p = 0.01), (F(3,22) = 2.2722, p < 0.05). RT decreased in PSD96 compared with CTRL (p = 0.02) and REST (p = 0.03), (F(3,21) = 2.9579, p < 0.05), similarly to –dT/dt (F(3,23) = 4.3408, p < 0.05). In addition, +dT/dt was lower in
Figure 3 – Effect of resistance training and paradoxical sleep deprivation on papillary muscle temporal parameters in terms of length-tension relationship. Two-way ANOVA with Duncan post-hoc test. Data expressed in mean ± standard error, with significance set at p = 0.05. TTP: time to develop tension peak; RT50%: time required for maximum developed tension to decrease by 50%; CTRL: control group; REST: resistance training group; PSD96: paradoxical sleep deprivation for 96 hours; REST/PSD96: resistance training followed by 96-hour paradoxical sleep deprivation; Lmax: maximum papillary muscle length. ‡ different from all groups in each stretching percentage tested

PSD96 (p = 0.02) and REST/PSD96 (p = 0.002) than in CTRL, and significantly different between RST/PSD96 and REST (p = 0.01) (F(1,18) = 4.6147, p < 0.05) (Figure 4).

No difference in time parameters was observed between the groups (F(3,23) = 0.7959, p < 0.05), except for RT50% at 60s in comparison with other rest times – 10 (p = 0.00004), 20 (p = 0.001), 30 (p = 0.004) and 120 seconds (p = 0.0006), (F(4,88) = 3.8313, p < 0.05) in REST/PSD96 (Figure 5).

**Contractile mechanics of papillary muscle in response to extracellular calcium**

There was an increase in DT in the REST group (F(1,18) = 5.1523, p = 0.02), and hence greater response to calcium-induced inotropy. RT was lower at Ca²⁺ 2.5 mM as compared with Ca²⁺ 1.5 mM levels in CTRL (p = 0.001) and REST/PSD96 (p = 0.04) (F(1,18) = 14.0730, p < 0.01), and lower at both Ca²⁺ 1.5 mM (p=0.009) and 2.5 mM (p = 0.02) in the PSD96 group than in CTRL group (F(3,18) = 3.2331, p < 0.05) (Figure 6).

Regarding +dT/dt, this variable was higher at Ca²⁺ 2.5 mM than Ca²⁺ 1.5 mM (p = 0.03) in the REST/PSD96 group, however, at both concentrations (Ca²⁺ 1.5 mM, p = 0.01 and Ca²⁺ 2.5mM, p=0.03), +dT/dt was lower in this group as compared with CTRL (F(1,18) = 9.354531, p < 0.01).

Similarly, reduced–dT/dt was observed at Ca²⁺ 1.5 mM and Ca²⁺ 2.5 mM in the PSD96 group as compared with CTRL (p = 0.03) and REST (p = 0.02), (F(3,29) = 3.6516, p < 0.05).

TTP increased at Ca²⁺ 2.5mM in PSD96 as compared with CTRL (p = 0.003), REST (p = 0.02) and REST/PSD96 (p = 0.01) (F(1,19) = 5.538, p < 0.05). No difference in RT50% was found between the groups (F(2,19) = 0.69, p < 0.05).

**Discussion**

Our results demonstrated the deleterious effect of PSD on myocardial contractility and the role of resistance training on partially attenuating such effect. Resistance training and PSD cause myocardial hypertrophy associated with reduced Lmax. This may be due to an increase in cardiac collagen content and reduced papillary muscle elongation resulting from an increase in corticosterone levels induced by PSD.

Baseline measures indicated depression of myocardial function in the groups subjected to PSD. Interestingly, these data are similar to those reported in previously infarcted or nephrectomized rats, strongly suggesting a marked effect of PSD on contractile and temporal parameters of papillary muscle in just 4 days.
Figure 5 – Effect of resistance training and paradoxical sleep deprivation on papillary muscle temporal parameters in terms of post-rest potentiation.
Two-way ANOVA with Duncan post-hoc test. Data expressed in mean ± standard error, with significance set at $p = 0.05$. TTP: time to developed tension peak; RT50%: time required for maximum developed tension to decrease by 50%; CTRL: control group; REST: resistance training group; PSD96: paradoxical sleep deprivation for 96 hours; REST/PSD96: resistance training followed by 96-hour paradoxical sleep deprivation; *different from CTRL; † different from the REST group; ‡ different from all other rest periods within the same group.

Figure 4 – Effect of resistance training and paradoxical sleep deprivation on papillary muscle contractile parameters in terms of post-rest potentiation.
One-way ANOVA with Duncan post-hoc test. Data expressed in mean ± standard error, with significance set at $p = 0.05$. DT: maximum developed tension; RT: isometric resting tension; $+dT/dt$: temporal variation of contraction force; $-dT/dt$: temporal variation of relaxation force; TTP: time to developed tension peak; CTRL: control group; REST: resistance training group; PSD96: paradoxical sleep deprivation for 96 hours; REST/PSD96: resistance training followed by 96-hour paradoxical sleep deprivation; *different from CTRL; † different from the REST group.
Temporal parameter of cardiac relaxation was increased in PSD96, possibly due to cardiac muscle myosin composition and calcium transient duration, both influenced by calcium signaling dysfunction. On the other hand, resistance training prevented the slowing down of relaxation time of papillary muscle, although it was not effective in preventing DT decrease.

The decrease in DT in the REST/PSD96 group may be explained by the high intensity of the resistance training. Nevertheless, although it promotes beneficial changes, high-intensity resistance training combined with PSD resulted in papillary muscle contractile function depression. No difference in DT between CTRL and REST was observed, suggesting preservation of contractile function of papillary muscle. In contrast, resistance training at pre-determined loads (60-70% of 1RM) has been shown to improve myosin ATPase activity and papillary muscle contractility. Regarding aerobic training, previous studies have shown higher DT in trained animals than in controls.

Analysis of contractile response of papillary muscles to stretching allowed the assessment of the Frank-Starling relationship. All groups, except the CTRL group, showed a depressed contractile response, suggesting a failure or exhaustion of the Frank-Starling mechanism in the myocardium. When +dT/dt was evaluated, a failure in this mechanism was detected in the PSD96 group only. Contractile response to stretching is generally related to the increase in the number of actin-related myosin heads associated with actin. Elongation of a muscle promotes the sliding and linkage between thick and thin filaments, which increases the number of cross-bridges and thereby the force generation. However, in the PSD96 group, the failure of the Frank-Starling mechanism may be due to other factors, such as changes in the calcium transient duration or myosin composition.
filaments, and consequent binding of Ca\(^{2+}\) to troponin. Therefore, it is plausible to say that the distance between the filaments is a determining factor for cardiac muscle physiological behavior.\(^{26,27}\)

The decrease in RT corroborates the lower contractile response in the PSD96 group in relation to the Frank-Starling mechanism, indicating stiffness and reduced contractility and relaxation capacity, as observed in infarcted animals.\(^{20}\) The lower the RT, the higher the muscle distension capacity, which is associated with increased collagen type III: collagen type I ratio.\(^{28}\) Another hypothesis is an impaired muscle oxygenation.\(^{29-31}\) Besides, similarly to baseline analysis, there was an increase in the relaxation time in the PSD96.

In our study, there was no increase in contractile parameters in the REST group. However, data on the literature have shown that regular exercise has an impact on length-tension relationship of left ventricular cardiomyocytes, thereby increasing contraction force.\(^{20,31}\) On the other hand, length-DT relationship was decreased in animals subjected to aerobic exercise, suggesting no effect of this type of exercise on attenuating this parameter.\(^{25}\)

Post-rest potentiation of electrical stimulations allows to indirectly determine the balance between the processes of Ca\(^{2+}\) reuptake, storage and release, controlled by sarcoplasmic reticulum, and intracellular Ca\(^{2+}\) release through the sarcolemma. A pause in electrical stimulation potentiates contraction, due to Ca\(^{2+}\) accumulation in sarcoplasmic reticulum during such pause, increased Ca\(^{2+}\) released by sarcoplasmic reticulum, and reduced Na\(^+\)/Ca\(^{2+}\) exchange activity in removing Ca\(^{2+}\) from the cells.\(^{32,33}\) In infarcted rats, post-rest potentiation was decreased in papillary muscle.\(^{20,25}\) These processes could be evidenced in our study when temporal variations of contraction (+dT/dt) and relaxation force (-dT/dt) were assessed in the PSD96 group, suggesting a possible kinetic dysfunction of Ca\(^{2+}\) associated with increased Na\(^+\)/Ca\(^{2+}\) exchanger activity.

Therefore, our findings suggest that PSD can affect cardiac function, and such effect is not prevented by high-intensity resistance training, since a +dT/dt depression was found in animals subjected to the exercise. It is possible that protocols of moderate- or low-intensity resistance training or even aerobic exercise would be more effective in improving contractile function of papillary muscle. Experimental data have shown an increased post-rest potentiation in infarcted rats submitted to aerobic training, which highlights the preventive role of this type of exercise.

Finally, we evaluated the contractile response of papillary muscle to increasing concentrations of Ca\(^{2+}\) in bathing solution. This approach aims to evaluate whether there is an improvement in contraction-relaxation behavior in response to increasing availability of Ca\(^{2+}\).\(^{34}\) Our results have shown that, in response to increasing Ca\(^{2+}\) concentrations, DT increased in the REST group and both RT and -dT/dt decreased in PSD96. The variable +dT/dt decreased in the REST/PSD96 group and temporal variation of cardiac contraction increased in the PSD96 group, suggesting an increase in the time to systole.

Altogether, these results suggest that both contractile and temporal response was negatively affected by PSD. In response to Ca\(^{2+}\), regardless of the ion concentration, there was an impaired response of papillary muscle of animals submitted to PSD. These findings are in accordance with those reported in infarcted animals.\(^{35}\)

## Conclusion

High-intensity resistance training was effective in improving temporal but not contractile parameters. Less intense exercise protocols may be more effective for myocardial protection, represented by the papillary muscle.

## Study limitations

The present study aimed to investigate the effects of sleep deprivation on myocardial contractility in the absence of associated comorbidities, which may represent confounding factors in clinical trials. For this reason, caution is needed in extrapolating these results to the clinical setting.

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Author contributions

Conception and design of the research: Mônico-Neto M, Antunes HKM. Acquisition of data: Giampá SQC, Mônico-Neto M, Souza HS, Portes LA, Serra AJ, Antunes HKM. Analysis and interpretation of the data: Giampá SQC, Mônico-Neto M, Portes LA, Serra AJ, Tucci PJF, Antunes HKM. Statistical analysis: Giampá SQC. Obtaining financing: Mello MT, Tufik S, Tucci PJF, Antunes HKM. Writing of the manuscript: Giampá SQC, Mônico-Neto M, Portes LA, Antunes HKM. Critical revision of the manuscript for intellectual content: Mônico-Neto M, Souza HS, Mello MT, Tufik S, Portes LA, Serra AJ, Tucci PJF, Antunes HKM.

Potential Conflict of Interest

No potential conflict of interest relevant to this article was reported.

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Study Association

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