Caffeine and physical training: effects on cardiac morphology and cardiovascular response

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Summary

Objective: to analyze the morphological structure of cardiac, blood pressure (BP), heart rate (HR) and heart rate variability (HRV) of rats subjected to physical training with supplementation of caffeine.
Methods: 60 rats were divided into 4 groups: control (CO), control with caffeine (CAF), trained control (TRE) and trained with caffeine (TCAF). All trained groups underwent 4 weeks of swimming, and all caffeine groups were supplemented by voluntary ingestion of caffeine diluted in drinking water.
Results: there were no changes to BP and HR between groups. Regarding HRV, there was a decrease in LFnorm (low frequency) and LF/HF ratio (low and high frequency) in TCAF and CAF compared to group ($p<0.02$ and $p<0.03$, respectively). An increase occurred in CAF compared to the CO in the component LFnorm ($p<0.05$). The results also showed an increase in the relative weight of heart in the TRE ($p<0.04$) and TCAF ($p<0.03$) compared to CO.
Conclusion: caffeine did not modify the hemodynamic responses. However, physical training resulted in a decrease in sympathetic response and an increase in relative heart weight.

Uniterms: caffeine; exercise; arterial blood pressure; heart rate.

Introduction

Caffeine, present in several food items ingested daily[1] can be used to increase physical performance[2], and effect that could be related to the antagonism of adenosine receptors in the central nervous system, increasing the release of dopaminergic neurotransmitters an affecting the perception of effort.[4] Furthermore, caffeine acts on the propagation of neural signals between the brain and the neuromuscular junction[5] and on the musculoskeletal system, facilitating stimulation and contraction.[6]

Some physical changes resulting from the use of caffeine are a reduction in bone density[7,8] and hemodynamic changes.[9,10] However, unlike structural bone changes (which could be compensated for with physical training, for example), hemodynamic changes[9,10] resulting from caffeine suggest greater precautions, such as increased blood pressure (BP) and heart rate (HR).[11,12] Therefore, although research may note an increase in parasympathetic activity in normotensives 30 minutes after the ingestion of 240 mg of caffeine[12], a study involving both healthy individuals and those with coronary diseases verified that caffeine promoted changes in cardiac microcirculation, characterized by a reduction in cardiac perfusion and increased coronary resistance.[13] We must also consider the differences between the effects of caffeine and substances that contain caffeine, such as coffee.[14]

However, the majority of the information about the effect of caffeine on the organism does not make an association with exercise. Physical training reduces the chance of cardiac diseases and, unlike caffeine, reduces peripheral vascular resistances and resting BP.[15] Considering that caffeine may cause cardiovascular changes when used long term, it is necessary to investigate its effects on this tissues when used chronically, in association with physical training protocols.

This study analyzed cardiac histology, BP, HR and HR variability (HRV) at rest in rats submitted to a period of physical training with caffeine supplementation.

Methods

Animals

60 male Wistar rats were used, weighting approximately 250 g, obtained from the Biological Sciences Center at the State University of Londrina. The animals were randomly separated into groups, kept in isolated cages at room temperature and a 12 hour light/dark cycle. Normocaloric feed (NUVILAB®) was provided and drinking water *ad libitum*. The study was approved by the Ethical Research Committee at the State University of Londrina (n. 28/10).

The animals were divided equally into four groups: control with caffeine (CAF), trained (TRE) and trained with caffeine (TCAF). The TRE and TCAS groups were submitted to physical training by swimming.

Caffeine supplementation

The CAF and TCAF groups were supplemented with 1 mg/mL of caffeine diluted in drinking water for 29 says, through voluntary ingestion[16], given that the use of gavage for long periods was shown to be harmful to the animals. The CO and TRE groups only received drinking water. The volume of liquid ingestion and consumption of feed were evaluated on the 1st, 10th and 20th day of training, with no statistical difference found among groups.

Physical training

The TRE and TCAF groups were submitted to 21 training sessions of swimming. The training was held five times per week at a moderate intensity[17] in individual tanks with water temperature between 28 and 32°C. The animals were submitted to a familiarization period with the aquatic environment for five days. During this period, the animals were placed in the tanks with 10 cm of water on the first day, gradually increasing every day until a depth of 30 cm was reached. From the sixth day of training, a load was added corresponding to approximately 3 to 5% of the animal's bodyweight, to adapt the intensity of the training. The load was adjusted using lead weights attaché to the animal's tail.

Verification of blood pressure and heart rate

Cannulation of the femoral artery and vein

The measurement of the BP and HR was realized via cannulation of the animals, which was conducted one day after the finalization of the training, in the morning.

Under anesthesia using pentobarbital (50 mg/mL), the animals were submitted to surgery to implant catheters in the femoral artery and vein. The vessels were dissected and polyethylene PE-10 catheters (4 to 5 cm) were attached to segments of polyethylene PE-50 (12 to 13 cm), previously filled with saline and anticoagulant (15 U/mL of heparin in saline solution) and obstructed with a metal pin. After implantation of the catheters, they were exteriorized in the dorsal region an attached to the skin with a surgical suture.

The arterial catheter was used to record the resting BP. After 24 hours of cannulation, permitting recovery from the effects of the anesthesia, the animals were submitted to recording of the baseline BP. They were kept in individual boxes for the entire post-operatory period and registration of the BP.

Blood pressure and heart rate recording

The direct recording of BP and HR was undertaken with the animals awake and in free movement. The arterial cannula of each animal was coupled to a pressure transducer (Powerlab MLT0380) and connected to a computerized registration system (Powerlab/ADInstruments). The results of the average BP (ABP) were recorded by the software itself. During the recording period the animals were kept in individual boxes in a quiet environment.

Spectral analysis of the HRV

The baseline recording of at least 10 minutes of HR recordings was submitted to analysis of the HRV in the frequency domain. The recordings were processed using the LA software (*Programma di Analisi Lineare, Universitá degli Studi di Milano*), which applies an algorithm to detect the inflection of cycle to cycle points on a periodic wave, determining the value of each beat of the systolic BP (SBP) and diastolic BP (DBP). Temporal series pulse intervals were also generated to measure the time interval between adjacent readings of diastolic pressure. The overall variability of pulse intervals was evaluated based on the variance of the temporal series. The variability of the pulse intervals in the domain of the frequency was evaluated using spectral analysis on the total variability components (VAR), low frequency (LF), high frequency (HF) and LF/HF (ratio between high and low frequency). The results were expressed in absolute figures (ms^2), normalized figures (norm) and percentage (%).

Euthanasia of animals

The animals were euthanized after 21 days of the exercise protocol, after the last measurement of the BP and HRV, via inhalation of a lethal dose of diethyl ether.

Histological analysis

After euthanasia of the animals, the heart was removed, washed in saline solution and weight on semi analytic scales. The heart was kept in 4% paraformaldehyde solution for 24 h, dehydrated in ethyl alcohol solutions at 70%, 90% and absolute alcohol. The material was then diaphonized in xylol and included in histological paraffin.

7 µm cuts were stained with hematoxylin / eosin. Images of 10 random fields were captured using the Motican imaging system (Motic Eletric Group Co. Ltd, Xiamen, China), and analyzed by three trained observers. The observers evaluated the 10 images. Therefore, for each heart, 10 images were analyzed by 3 observers, totaling 30 analyses. The number of fields with areas of necrosis and the presence of myocardial inflammatory infiltrate were observed. Areas of necrosis of the myocardium presented cardiac muscular fibers with loss of striations, acidophilic cytoplasm and absence of nuclei. Inflammatory infiltrate was observed, such as the presence of neutrophils and macrophages phagocytizing necrotic fibers or the interstitium of heart tissue.

Statistical treatment

The data was analyzed using descriptive methods, expressed in averages and standard deviations. The normality of the variables was analyzed using the Shapiro-Wilk test. In the comparison between groups, the Levene test was used to verify the homogeneity of the sample. As the data was homogeneous, the ANOVA One-Way and post hoc LSD test were used. When the homogeneity of the data was not proven, the corresponding nonparametric Kruskall-Wallis test was used. Differences between the groups were considered significant when $p<0.05$. The Chi Squared test with Yates correction was used to detect differences in frequencies of the lesions between the groups.

Results

Differences were not noted in the resting ABP and HR values among the groups after the experimental protocol. In relation to the HRV, there was a reduction in the components of LFnorm in the LF/HF ratio in the TCAF group in relation to the CAF group ($p<0.02$ and $p<0.03$, respectively). The CAF group presented an increase in the LFnorm component ($p<0.05$) in relation to the CO group. There was no difference in the other HRV components among the groups (Table1).

In relation to weight (body and heart) no difference was noted in the weight of the animals and the absolute weight of the heart among the groups studied. However, an increase in weight was noted in relation to the heart in the TRE ($p<0.04$) and TCAF ($p<0.3$) groups compared to the CO group.

However, there was also an increase in the TCAF group only in relation to the CAF group ($p<0.05$). There was no difference in the areas of edema, inflammatory infiltrate and necrosis in the striated muscle among the groups. Figure 1 shows the histological photos of the same heart fragment for the trained (TRE and TCAF) and control groups (CO and CAF), in which there was no difference in cardiac morphology among the groups.

TABLE 1 Body weight, heart weight, relative weight of the heart after euthanasia, average blood pressure (ABP), heart rate (HR) and heart rate variation (HRV) component at rest in the different types of group (average ± standard error)

Variables	CO	CAF	TRE	TCAF
Animal weight (g)	363.1±9.32	334.6±10.1	337.3±9.50	326.2±8.66
Heart weight (g)	1.07± 0.64	1.04±0.35	1.09±0.45	1.18±0.68
Relative heart weight (%g)	0.29±0.14	0.31±0.07	0.33±0.01 *	0.35±0.02 * †
ABP (mmHg)	108.7±3.91	107.4±3.93	100.7±9.95	106.4±8.64
HR (bpm)	363.5±13.9	383.9±21.9	328.2±23.6	408.6±33.6
VAR ms^2	8.16±1.78	15.7±6.82	7.99±3.28	4.05±0.88
LF ms^2	0.78±0.49	4.13±2.50	1.29±0.64	0.14±0.07
HF ms^2	3.85±0.93	6.70±2.90	3.62±2.04	2.22±0.44
LF%	12.7±5.09	25.3±6.15	21.8±10.5	3.47±1.58
HF%	67.8±7.22	57.4±6.50	57.5±9.53	76.4±3.48
LFnorm	9.7±7.39	29.3±6.78*	24.0±10.9	4.12±1.80†
HFnorm	49.8±21	70.6±6.78	75.9±10.9	95.8±1.8
LF/HF	0.25±0.13	0.59±0.19	0.45±0.27	0.05±0.02†

CO = control group; CAF = caffeine group; TRE = trained group; TCAF = trained group with caffeine; VAR = total variability; LF = low frequency; HF = high frequency; LF/HF = ratio between low and high frequency; norm = normalize values; ms^2 = absolute values; * = significant difference in relation to CO ($p<0.05$); † = significant difference in relation to CAF ($p<0.05$).

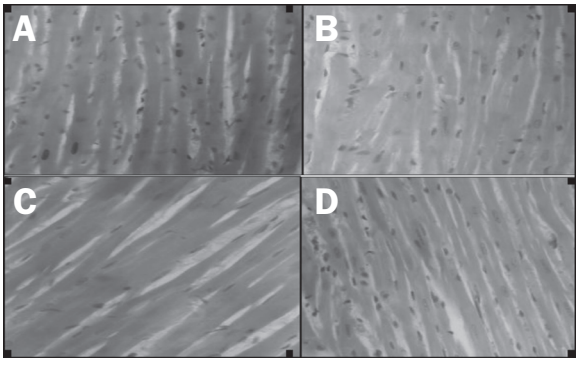

FIGURE 1 Images of the same location of histological cuts to the heart in different types of group. **(A)** CO = control group; **(B)** CAF = caffeine control group; **(C)** TRE = trained control group; **(D)** TCAF = caffeine trained group.

Discussion

The results do not show an increase in the ABP and HR after chronic supplementation with caffeine. However, experiments using rats have demonstrated that chronic supplementation with caffeine may cause physiological adaptations such as decreased sensitivity and down-modulation of adenosine receptors.[16] Thus, the consumption of caffeine may act on possible regulatory mechanisms for BP and HR due to its antagonistic effect on adenosine receptors. Among the mechanisms involved, there may be inhibition of phosphodiasterase, activation of the sympathetic nervous system (via release of catecholamine from the adrenal medulla), stimulation of the adrenal cortex (release of corticosteroids) and effects on the urinary system (diuresis, natriuresis and activation of the renin-angiotensin-aldosterone system).[18]

Some studies have confirmed the results obtained in the present study in relation to the ABP in the group that ingested caffeine (CAF). For example, Kost et al.[19] did not find a change in the ABP after 15 days of consuming 1 mg/mL of caffeine. In humans, Debrah et al.[20] did not find a change in the ABP after consumption of caffeine for a week (250 mg), either. The possible mechanisms involved in maintaining the ABP, even with the administration of caffeine, are related to the chronic effect of this substance. In this case, it is said that the use of caffeine may not lead to an increase epinephrine and norepinephrine, i.e. the sympathetic system was not adjusted to lead to an increase in ABP. The maintenance of the ABP in the trained group (TRE) would be expected after a training protocol in normotensive rats.[21] Medeiros et al.[21] also did not find any changes in ABP after swimming training for 8 weeks (60 min/day, 5 times per week) with the load increased until reaching 5% of the bodyweight. These results may be related to the baseline BP level, as it appears that a hypertensive sample would benefit more from the physical effect on the reduction of BP.[22]

Similarly to the ABP, no changes were observed in the HR. However, the HR may behave differently when the individual is exposed to caffeine. As an example, Kost et al.[19] did not find any HR changes after 1mg/mL consumption of caffeine over 15 days. On the other hand, White and Nguyen[23] noted an increase in HR after 15 days of caffeine consumption at 2 mg/mL. This HR response appears to be related to the caffeine dose used, as a large part of the research on rats has used a dose of 1 mg/mL of caffeine. However, various studies with humans [3,20,24,25] did not find any HR changes after consumption between 250 and 450 mg of caffeine in periods varying from six days to three months. This may be related to the baroreflex effect that the HR suffers as a result of BP adjustments, as when there is a change in this variable, the HR may be adjusted as a result of the sympathetic or parasympathetic modulation of the heart.[26]

Although the ingestion of caffeine may alter the baroreflex responses through antagonism of adenosine, modifying the responses in the autonomous nervous system, with an increase in sympathetic responses to the heat and culminating in an increased HR[26], some experiments[9,27,28] have found a reduction in HR after consumption of 300 to 445 mg of caffeine, during 1 to 12 weeks. This reduction in HR may be associated to cardiovascular adjustments in an attempt to compensate for the increased BP. Nevertheless, the mechanisms that could explain such results are not yet clear.

In this study, the autonomic behavior of the heart demonstrated changes in the HRV in response to exercise and supplementation with caffeine. The TCAG group obtained a reduction in the LFnorm (sympathetic) component and in the LF/HF ratio. This finding suggests that the association between aerobic training and caffeine may have caused adjustments to the parasympathetic modulation of the heart. After a physical training period, there is an increase in the HF (parasympathetic) component, and a reduction or maintenance of the LF component in sedentary humans.[29]

Few studies have analyzed the chronic effect of caffeine on HRV. Hibino et al.[12] analyzed the autonomic modulation of the heart after acute ingestion of 240 mg of caffeine over 30 minutes, and observed an increase in parasympathetic activity (through spectral analysis of the HR variability) in normotensive humans. That is, acute ingestion of caffeine caused an increase in the parasympathetic response of the heart. However, in the present study, the chronic autonomic response in the CAF group showed an increase in the LFnorm (sympathetic) component. This may have occurred due to the antagonistic effect of caffeine on adenosine, increasing circulating catecholamine (norepinephrine), resulting in increased activity of the sympathetic nervous system.

In the present study, in relation to aerobic training, the rats trained at a low to moderate intensity with loads at 3 to 4% of their bodyweight. Gobatto et al.[17], investigating young, untrained rats, demonstrated that loads of 5 to 6% of the bodyweight represented a balanced between the production and removal of blood lactate, being considered as loads with aerobic components. The present study did not evaluate blood lactate collection. However, as the loads imposed on the rats in training were at a lower range than 6% of the bodyweight, it

can be speculated that the rats were undertaking aerobic training at the intensity proposed by the study. Therefore, in addition to hemodynamic and autonomic changes, chronic physical exercise with a low to moderate intensity may cause adaptations in the cardiovascular system. Some studies have demonstrated an increase in the mass and volume of the heart as a result of overload imposed by exercise.[21,30] In the present study there was an increase in weight relating to the heart in the trained groups. The data from Cunha et al.[30] corroborates the findings of the present study. The authors found an increase in the relative weight of the heart in elderly rats trained through swimming for eight weeks (30 min/day, 5 times per week) with a load of 5% of their bodyweight. These adaptations may be explained by the effect that physical exercise causes on the heart, causing an adaptive process to occur, with a consequent increase in the thickness of ventricular walls, seeking to compensate the stress imposed on the organ. This cardiac adaptation may provide increased efficiency of the cardiovascular system, increasing the supply of oxygen to muscles during effort.[31,32] However, it can be speculated that the relative weight of the heart is higher because trained animals present a lower body mass.

Nevertheless, histological and morphological changes were not identified in the heart in the present study. Even with chronic consumption of caffeine, the structures of the heart remained unchanged, with absence of necrosis, neutrophils and maintenance of the fibers. One of the adaptations to chronic training is the reduction of inflammatory mediators and quantitative changes in cardiac muscular cells, vessels and interstitial tissue in the myocardium.[33] In the results of the present study, changes caused by training the heart of the rats were not observed.

Regardless of our conclusions, there are limitations that should be commented on. For operational reasons, blood lactate was not collected and, lastly, histological processes of the blood vessels were not conducted.

Conclusion

Our results suggest that the chronic use of caffeine does not change hemodynamic responses. However, the responses to sympathetic-vagal balance may suffer the influence of caffeine due to an increase in sympathetic response (LFnorm) by the heart. The adaptations suffered due to physical training result in decreased sympathetic response (LFnorm and LF/HF) and an increase in the relative weight of the heart, confirming that physical exercise has a beneficial role in cardiovascular adjustments even at low to moderate intensity.

In the present study, signs of cardiotoxicity were not identified, but we cannot affirm that caffeine is exempt for cardiotoxicity in other experimental conditions.

Resumo

Cafeína e treinamento físico: efeitos na morfologia cardíaca e respostas cardiovasculares

Objetivo: analisar a histologia cardíaca, a pressão arterial (PA), a frequência cardíaca (FC) e a variabilidade da frequência cardíaca (VFC) de ratos submetidos a treinamento físico e suplementação de cafeína.

Métodos: sessenta ratos foram divididos em grupos controle (CO), controle suplementado com cafeína (CAF), treinados controle (TRE) e treinados suplementados com cafeína (TCAF). Os grupos de treinamento realizaram natação por quatro semanas, e os grupos de cafeína foram suplementados por ingestão voluntária de cafeína diluída em água.

Resultados: não houve modificações para PA e FC entre os grupos. Em relação à VFC, houve diminuição nos componentes LFnorm (baixa frequência) e LF/HF (razão baixa e alta frequência) em TCAF em relação a CAF ($p<0,02$ e $p<0,03$, respectivamente). Houve também aumento em CAF em relação a CO no componente LFnorm ($p<0,05$). Os resultados também mostraram aumento no peso relativo do coração em TRE ($p<0,04$) e TCAF ($p<0,03$) em relação a CO.

Conclusão: a cafeína não modificou as respostas hemodinâmicas. Entretanto, a natação diminuiu a resposta simpática e aumentou o peso relativo do coração.

Unitermos: cafeína; exercício; pressão arterial; frequência cardíaca.

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