Universidade Federal do Rio Grande do Sul

Faculdade de Farmácia

Disciplina de Trabalho de Conclusão de Curso de Farmácia

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Orientadora

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Aos meus pais, ***Olavio*** *e* ***Mariza****, pela confiança, incentivo e amor incondicional.*

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“Um homem precisa viajar. Por sua conta, não por meio de histórias, imagens, livros ou TV. Precisa viajar por si, com seus olhos e pés, para entender o que é seu. Para um dia plantar as suas próprias árvores e dar-lhes valor. Conhecer o frio para desfrutar o calor. E o oposto. Sentir a distância e o desabrigo para estar bem sobre o próprio teto. Um homem precisa viajar para lugares que não conhece para quebrar essa arrogância que nos faz ver o mundo como imaginamos, e não simplesmente como é ou pode ser; que nos faz professores e doutores do que não vimos, quando deveríamos ser alunos, e simplesmente ir ver.”

Amyr Klink

Resumo

O metilfenidato, psicoestimulante amplamente utilizado no tratamento do transtorno de déficit de atenção/hiperatividade, pode afetar adversamente o funcionamento cardíaco, porém os mecanismos envolvidos ainda não são conhecidos. Baseado nisso, o objetivo desse estudo foi determinar alguns parâmetros de estresse oxidativo em coração de ratos submetidos à administração aguda e crônica de metilfenidato. Ambos os modelos foram induzidos em ratos Wistar pela administração intraperitoneal de metilfenidato na dose de 2,0 mg/Kg de peso corporal. Os animais controles receberam solução salina nos mesmos volumes. No modelo agudo, ratos com 45 dias de vida receberam uma única administração de metilfenidato; no modelo crônico os ratos receberam metilfenidato, uma vez ao dia, do 15º ao 45º dia de vida. Os resultados mostraram que uma única administração de metilfenidato altera os níveis de nitrito e alguns parâmetros de dano a biomoléculas, como TBARS (índice de peroxidação lipídica), carbonilas e TNB (referente a dano proteico) em coração de ratos. Por outro lado, no coração de ratos tratados cronicamente com metilfenidato, não houve alterações nos níveis de nitrito e nos parâmetros de dano a biomoléculas, mas observamos um aumento na produção de espécies reativas medida pelo DCF formado. Em resumo, esse estudo apresenta uma nova abordagem sobre esse assunto, a partir da evidência do envolvimento do estresse oxidativo, pelo menos em parte, nos efeitos cardiovasculares associados ao uso de metilfenidato.

Palavras-chave: estimulante do sistema nervoso central; metilfenidato; toxicidade cardiovascular; produção de radicais livres, estresse oxidativo/nitrativo; estudo experimental.

Estes resultados serão ampliados e escritos na forma de artigo científico para publicação em revista especializada.

Evidence that methylphenidate promotes redox imbalance in heart of rats

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Abstract

Methylphenidate, a psychostimulant amply used in treatment of attention deficit/hyperactivity disorder, can adversely affect the functioning of the heart, but the mechanisms involved are unknown. Based on this, the aim of the present study was to evaluate some parameters of oxidative stress in heart of rats subjected to acute and chronic methylphenidate administration. Both models were induced in Wistar rats by intraperitoneal administration of methylphenidate in dose of 2.0 mg/Kg of body weight. Controls received saline in the same volumes. In acute model, rats with 45 days of life received a single administration of methylphenidate; in chronic model, rats received methylphenidate administration once a day, from the 15th to the 45th day of life. Results showed that a single administration of methylphenidate alters nitrite levels and some parameters of damage to biomolecules in heart of rats. On the other hand, in the heart of rats chronically treated with methylphenidate, no changes in the levels of nitrite and the parameters of damage to biomolecules, but observed an increase in reactive species production measured by DCF formed. In summary, this study presents a new approach on this subject, from the evidence, of the involvement of oxidative stress, at least in part, in cardiovascular effects associated with the use of methylphenidate.

Keywords: central nervous system stimulant; methylphenidate; cardiovascular toxicity; free radical production; oxidative/nitrative stress; experimental study.

Introduction

Attention deficit/hyperactivity disorder is the main neurobehavioral disorder among children and adolescents and their primary symptoms are inattention, impulsivity and hyperactivity [1, 2]. Treatment of this disorder involves a multipronged approach, being that the more frequents are medications such as atomoxetine, a selective norepinephrine reuptake inhibitor, stimulant-class medications such as amphetamines and pemoline. Nevertheless, methylphenidate (Ritalin®, MPH), a psychostimulant with that blocks both dopamine and norepinephrine transporter, is the mainstay of treatment for attention deficit/hyperactivity disorder [3-6].

Although MPH may be clinically effective and safe in treating symptoms of attention deficit/hyperactivity disorder, the high increase in the frequency of prescriptions in recent decades, especially for young adults and children, including preschoolers of 2-4 years old [7, 8], triggers a concern about their cardiovascular safety [6, 9-12]. It has been described that systolic and diastolic blood pressure and heart rate increase in children and adults treated with MPH [11, 13], a fact that could contribute to myocardial infarction, sudden cardiac death and stroke [14]. Although little is known about the mechanisms involved in the development of cardiomyopathy related to the use of stimulant drugs in adolescents and children, it has been suggested that free radical production/oxidative stress may play a role in these cardiac changes [10].

Reactive oxygen species and reactive nitrogen species are normally produced in processes of biological oxidation and can play both beneficial and deleterious role. They have beneficial role when working in the activation of immune cells to fight invading microorganisms, and deleterious role when leading to damage of biomolecules such as lipids, proteins and DNA that can result in loss or alteration of

their functions [15]. Under physiological conditions the reactive species are maintained in balance due, in large part, to the neutralization capacity of the enzymatic and/or non-enzymatic antioxidant defense systems. Superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) compose antioxidant enzymatic defenses. Non-enzymatic are represented by ascorbic acid, α-tocopherol, glutathione, carotenoids, flavonoids and others. An imbalance between the production of reactive species and the antioxidant defense capacity tissue can induce oxidative stress, which can provoke tissue alterations, such as adaptation (if not too severe, since it usually results in up-regulation of the synthesis of antioxidant defense systems in order to restore balance oxidant/antioxidant), cellular injury (which involves damage to biomolecules in the body), or cellular death by necrosis or apoptosis, in severe oxidative stress [16].

Considering that MPH has been used extensively by young adults and children and, to our knowledge, there is a lack of studies that investigate the mechanisms involved in cardiomyopathies promoted by MPH, the purpose of this study was to investigate the effect of acute and chronic MPH administration on parameters of oxidative stress, namely reactive species production [2′7′-dichlorofluorescin diacetate (H_2DCF-DA), nitrite levels], antioxidant defences [total radical trapping antioxidant potential (TRAP) and activities of antioxidant enzymes (SOD, CAT, GPx) and SOD/CAT ratio], as well as on parameters of biomolecule damage [thiobarbituric acid reactive substances (TBARS levels), carbonyl content and equivalent to the amount of sulfhydryl groups (TNB levels)] in heart of rats.

Materials and methods

Animals

Wistar rats were obtained from the Central Animal House of the Department of Biochemistry of the Federal University of Rio Grande do Sul, Porto Alegre, Brazil. Animals were maintained on a 12:12 hours light/dark cycle (lights on 07:00–19:00 h) at a constant temperature (22± ºC) colony, with free access to water and 20% (w/w) commercial protein chow. Animal care followed the NIH ‘‘Guide for the Care and Use of Laboratory Animals’’ (NIH publication no. 80–23, revised 1996).

Acute MPH administration

Wistar rats (n = 24) with 45 days of life were divided in three groups. First and second groups received a single intraperitoneal administration of MPH at a dose of 2.0 mg/kg of body weight, resulting in plasma and brain levels of MPH within the range achieved under clinical conditions in humans [17, 18]. First group was beheaded without anaesthesia two hours after the administration, while the second group was beheaded twenty four hours after the administration. Based on pharmacokinetics studies that show that MPH presents maximum plasma concentration at 1 to 3 hours after administration and a t½ of approximately 2.6 to 3 h, being rapidly metabolized and excreted in urine [19], we chose at 2 and 24 hours in order to investigate the direct and indirect effect, respectively, of MPH on the heart. Third group (control group) received equivalent volume of 0.9 % saline solution. The heart was obtained for evaluating of oxidative stress parameters.

Chronic MPH administration

Wistar rats (n = 24) were divided in three groups and received intraperitoneal administrations, once a day, from the 15th to the 45th day of life. First and second

groups received MPH in dose 2.0 mg/kg of body weight. First group was beheaded without anaesthesia two hours after the last administration, while the second group was beheaded twenty four hours after the last administration. Third group received equivalent volume of 0.9 % saline solution (control group). The heart was obtained for evaluating of oxidative stress parameters. We chose to start administering MPH to rats at 15 days of life because that period has characteristics that correspond to the development of children [20], whose use of MPH has increased and is quite worrying. The treatment was ended at 45 days of life because it has been shown that 28 days of treatment in the life cycle of the rat mimics the chronic treatment in humans [21].

Tissue and homogenate preparation

The hearts were homogenized in 10 volumes (1:10, w/v) of 20 mM sodium phosphate buffer, pH 7,4 containing 140 mM KCl. Afterwards, the homogenates were centrifuged at 800 x g for 10 minutes at 4 ºC. The pellet was despised to discard nuclei and cell debris and the supernatant was taken to biochemical assays.

DCF formed determination

Reactive species production was measured following a method based in the oxidation of 2′7′-dichlorofluorescin (H_2DCF) [22]. Samples (60 µL) were incubated for 30 minutes at 37 ºC in the dark with 240 µL of 100 µM 2′7′-dichlorofluorescin diacetate (H_2DCF-DA) solution in a 96-well plates. H_2DCF-DA is cleaved by intracellular esterases to form non-fluorescent H_2DCF, which is rapidly oxidized by some reactive oxygen and/or nitrogen species present in samples, producing the highly fluorescent compound dichlorofluorescin (DCF), which can be measured at

488 nm excitation and 525 nm emission. A calibration curve was performed with standard DCF (0.25–10 mM) and the levels of reactive species were calculated as nmol DCF formed per milligram of protein.

Nitrite levels determination

Nitrite is the stable endproduct of the autoxidation of nitric oxide in aqueous solution [23]. Nitrite levels were measured using the Griess reaction; 100 µL of sample was mixed with 100 µL Griess reagent (1:1 mixture of 1 % sulfanilamide in 5 % phosphoric acid and 0.1 % naphthylethylenediamine dihydrochloride in water) and incubated in 96-well plates for 10 minutes at room temperature. The absorbance was measured on a microplate reader at a wavelength of 543 nm. Nitrite concentration was calculated using sodium nitrite standards [24]. The results were reported as µmol of nitrite per milligram of protein.

SOD activity assay

This method for the assay of SOD activity is based on the capacity of pyrogallol to autoxidize, a process highly dependent on anion superoxide which is a substrate for SOD [25]. Briefly, to 15 µL of each sample were added 215 µL of a mixture containing 50 mM Tris buffer pH 8.2 with 1 mM EDTA and 30 mM CAT. After that, 20 µL of pyrogallol were added and the absorbance was immediately recorded each 30 seconds for 3 minutes at 420 nm in SpectraMax M5/M5 Microplate Reader. The inhibition of autoxidation of pyrogallol occurs in the presence of SOD, whose activity can be then indirectly assayed spectrophotometrically. A calibration curve was performed with purified SOD as standard, in order to calculate the activity of SOD present in the samples. One SOD unit is defined as the amount of SOD

necessary to inhibit 50 % of pyrogallol autoxidation and the specific activity is reported as units per milligram of protein.

CAT activity assay

Catalase activity was assayed using SpectraMax M5/M5 Microplate Reader. The method used is based on the disappearance of hydrogen peroxide at 240 nm in a reaction medium containing 20 mM hydrogen peroxide, 0.1 % Triton X-100, 10 mM potassium phosphate buffer pH 7.0 [26]. One CAT unit is defined as one mmol of hydrogen peroxide consumed per minute and the specific activity is calculated as units per milligram of protein.

GPx activity assay

Glutathione peroxidase activity was measured using tert-butyl-hydroperoxide as substrate [27]. NADPH disappearance was continuously monitored with using SpectraMax M5/M5 Microplate Reader at 340 nm for 4 minutes. The medium contained 2 mM glutathione, 0.15 U/mL glutathione reductase, 0.4 mM azide, 0.5 mM tertbutyl-hydroperoxide, and 0.1 mM NADPH. One GPx unit is defined as one mmol of NADPH consumed per minute and the specific activity is represented as units per milligram of protein.

TRAP determination

Total radical-trapping antioxidant potential (TRAP) was determined by measuring the chemiluminescence intensity of luminol induced by 2,2'-azo-bis-(2-amidinopropane) (ABAP) thermolysis in Perkin-Elmer Microbeta Microplate Scintillation Analyzer [28, 29]. Two hundred and forty microliters of a system containing ABAP (10 mM) dissolved in 50 mM sodium phosphate buffer pH 8.6 plus

luminol (5.6 mM), was added to a microplate and the initial chemiluminescence was measured. Ten microliters of 300 µM Trolox (water-soluble α-tocopherol analogue, used as a standard) or 10 µL of sample was added to each plate well, producing a decrease in the initial chemiluminescence value. This value is kept low, until the antioxidants present in the sample are depleted, when chemiluminescence returns to its initial value. The time spent by the sample to keep chemiluminescence return to its low initial value is directly proportional to the antioxidant capacity of the tissue. The results were represented as nmol Trolox per milligram of protein.

TBARS levels determination

Thiobarbituric acid reactive substances (TBARS) levels, an index of lipid peroxidation, were determined according to the method described by Ohkawa et al. [30]. Briefly, 50 µL of 8.1 % sodium dodecyl sulfate, 1.5 mL of 20 % acetic acid solution adjusted to pH 3.5, 1.5 mL of 0.8 % thiobarbituric acid, and 700 µL of distilled water were added to 500 µL of tissue homogenate in a Pyrex tube. The mixture was vortexed and the reaction was carried out in a boiling water bath for 1 hour. After cooling with tap water, the mixture was allowed to cool on water for 5 minutes, centrifuged at 750 x g for 10 minutes, and the resulting pink color was determined in a spectrophotometer at 535 nm. A calibration curve was generated using 1,1,3,3-tetramethoxypropane as a standard. The results were reported as nmol of TBARS per milligram of protein.

Carbonyl content determination

Oxidatively modified proteins present an enhancement of carbonyl content [31]. In this study, protein carbonyl content was assayed by a method based on the

reaction of protein carbonyls with dinitrophenylhydrazine forming dinitrophenylhydrazone, a yellow compound, measured spectrophotometrically at 370 nm [32]. Briefly, 100 µL of homogenate were added to plastic tubes containing 400 µL of 10 mM dinitrophenylhydrazine (prepared in 2 M HCl). This was kept in the dark for 1 hour and vortexed each 15 minutes. After that, 500 µL of 20% trichloroacetic acid were added to each tube. The mixture was vortexed and centrifuged at 20.000 x g for 3 minutes. The supernatant obtained was discarded. The pellet was washed with 1 mL ethanol/ethyl acetate (1:1 v/v), vortexed, and centrifuged at 20.000 x g for 3 minutes. The supernatant was discarded and the pellet re-suspended in 600 µL of 6 M guanidine (prepared in a 20 mM potassium phosphate solution pH 2.3). The sample was vortexed and incubated at 60 ºC for 15 minutes. After that, it was centrifuged at 20.000 x g for 3 minutes and the absorbance was measured at 370 nm (UV) in a quartz cuvette with a Hitachi U-2001 double-beam spectrophotometer with temperature control. Results were represented as nmol of carbonyl content per milligram of protein.

TNB levels determination

This assay was performed as described by Aksenov and Markesbery [33], which is based on the reduction of 5,5'-dithio-bis(2-nitrobenzoic acid) (DTNB) by sulfhydryl groups, which in turn become oxidized (disulfide), generating a yellow derivative (TNB) whose absorption is measured spectrophotometrically at 412 nm. Briefly, 15 µL of homogenate were added to 275 µL of phosphate buffer saline pH 7.4 containing 1 mM EDTA. The reaction was started by the addition of 10 µL of 10 mM DTNB and incubated for 30 minutes at room temperature in a dark room. Results were reported as nmol of TNB per milligram of protein.

Protein determination

Protein was measured according to Lowry et al. [34] for all techniques. Serum bovine albumin was used as standard.

Statistical analysis

The data were analyzed by one-way analysis of variance (ANOVA) followed by Tukey test when F value was significant and are expressed as mean standard deviation. All analyses were performed using the Statistical Package for the Social Sciences (SPSS) software in a PC compatible computer. Differences were considered statistically significant when $p < 0.05$.

Results

Effect of MPH administration on reactive species levels

Initially, we investigated the effect of acute and chronic MPH administration on DCF formed and nitrite levels, which measure reactive species levels and the product of the metabolism of nitric oxide, respectively. Figure 1A shows that acute MPH administration did not affect reactive species levels in heart of rats at 2 hours, but increased at 24 hours after the last administration of this drug [$F(2,19) = 13.631$; $p < 0.001$]. Figure 1A also shows that chronic MPH administration increased DCF formed from the oxidation of H_2DCF at 2 and 24 hours after the last administration of MPH [$F(2,19)= 9.689$; $p < 0.01$], suggesting that MPH provokes an increase in reactive species levels in heart of rats. As it can be observed in Figure 1B, the levels of nitrite were increased at 2 and 24 hours [$F(2,21) = 57.750$; $p < 0.001$] after a single MPH administration. On the other hand, nitrite levels were not altered in heart of rats

submitted to chronic MPH administration at 2 and 24 hours after the last administration [$F(2,19) = 2.323$; $p > 0.05$], when compared to control group.

Effect of MPH administration on enzymatic and non-enzymatic antioxidant defenses

For a more precise analysis of equilibrium redox is necessary to evaluate the enzymatic and non-enzymatic antioxidant system, since changes in this system can prevent damage to cellular components promoted by an increase in reactive species production. Thus, we investigated the effect of MPH on enzymatic defenses (Figure 2). As it can be observed in Figure 2A, acute MPH administration did not alter SOD activity in heart of rats in any tested time [$F(2,16) = 0.156$; $p > 0.05$], but in chronic MPH administration the activity of this enzyme was increased in heart of rats at 24 hours after the last injection of MPH [$F(2,19) = 5.043$; $p < 0.05$]. Figure 2B shows that acute MPH administration increased CAT activity at 24 hours, but not at 2 hours, after the administration of MPH [$F(2,16) = 4.996$; $p < 0.05$] when compared to control group. On the other hand, chronic MPH administration did not alter CAT activity in heart of rats in any time evaluated [$F(2,19) = 3.062$; $p > 0.05$]. In addition, acute administration of MPH decreased SOD/CAT ratio in heart of rats at 24 hours, but not at 2 hours, after a single MPH administration [$F(2,16) = 3.722$; $p < 0.05$], while chronic administration of MPH did not alter SOD/CAT ratio in any time evaluated [$F(2,19) = 2.426$; $p > 0.05$] (Figure 2C) . GPx activity was increased in heart of rats at 2 and 24 hours after acute MPH administration [$F(2,21) = 7.331$; $p < 0.01$], but in contrast, the activity of this enzyme was decreased in heart of rats submitted to chronic MPH administration at 2 and 24 hours after the last administration of this drug [$F(2,19) = 8.116$; $p < 0.01$] (Figure 2D).

Furthermore, we also assessed the effect of acute and chronic MPH administration on the total antioxidant capacity non-enzymatic, namely TRAP (Figure 3). TRAP was not changed in heart of rats submitted to acute MPH administration in any time evaluated [$F(2,15)= 0.976$; $p > 0.05$], but increased in heart of rats submitted to chronic MPH administration at 2 and 24 hours after the last administration of MPH [$F(2,14) = 23.331$; $p < 0.001$], when compared to control group.

Effect of MPH administration on parameters of lipid and protein damage

Since it has been demonstrated that changes in levels of reactive species and antioxidant defenses promote damage to biomolecules in heart of rats [35], in the present study we also investigated the effect of acute and chronic MPH administration on TBARS, a measure of lipid damage, as well as on carbonyl content and TNB levels, which measure protein damage. Figure 4A shows that acute MPH administration increased TBARS levels in heart at 2 and 24 hours after a single administration [$F(2,17) = 7.842$; $p < 0.01$], while chronic MPH administration did not affect TBARS levels in heart of rats any time evaluated [$F(2,19) = 1.767$; $p > 0.05$]; Figure 4B showed that carbonyl content was not affected at 2 hours, but was increased at 24 hours in heart of rats subjected to acute MPH administration [$F(2,21) = 14.089$; $p < 0.001$]; on the other hand, chronic MPH administration did not affect carbonyl content in heart of rats in any time evaluated [$F(2,19) = 0.620$; $p > 0.05$] (Figure 4B). TNB levels were increased in heart of rats at 2 and 24 hours after a single MPH administration [$F(2,21) = 21.599$; $p < 0.001$], but chronic MPH administration did not alter TNB levels in heart of rats in any time evaluated [$F(2,19) = 0.909$; $p > 0.05$] when compared to their respective control group (Figure 4C).

Discussion

Attention deficit/hyperactivity disorder is recognized as a common neurobehavioral disorder whose diagnosis has increased greatly in recent years [36, 37] and it is estimated a worldwide prevalence of 5.29 % [38]. It has been shown a large increase in the incidence and prevalence of patients with attention defcit/hyperctivity disorder [39], as well as widespread use of stimulant medications for treatment, especially MPH [3-5,40]. Recently, relevant scientific studies have raised concerns about the cardiovascular safety of patients treated with MPH and there are data showing that this medication can affect the cardiovascular system [6, 9-12], but such mechanisms are not clear.

Using an experimental model in which rats received the same dose of MPH amply used in the clinic, we have previously showed that MPH administration induces changes in oxidative stress parameters in encephalic structures and blood [41, 42].

Free radicals are extremely harmful to the heart and recently studies show their involvement with many aspects of cardiovascular disease [43-45]. Considering that the mechanisms involved in cardiomyopathies observed in MPH users are still poorly understood and that evidences show that oxidative stress may be responsible for these effects, in the present study our goal was evaluated some parameters of oxidative stress in heart of rats decapitated at 2 or 24 hours after acute or chronic MPH administration, in order to evaluated the effect direct and indirect of experimental design, respectively.

Results show that animals submitted to an acute MPH administration presented an increased in the levels of DCF formed at 24 hours, but not 2 hours,

after the administration of MPH. SOD and CAT activities, as well as SOD/CAT ratio were not altered at 2 hours, but CAT activity was increased and SOD/CAT ratio was decreased at 24 hours after acute MPH administration in heart of rats. GPx activity increased at 2 and 24 hours after a single administration of MPH in heart of rats. TRAP was not altered in any time tested, while TBARS and TNB levels, but not carbonyl content, were increased in heart of rats at 2 hours after a single administration of MPH. It was observed an increase in TBARS and TNB levels, as well as in carbonyl content in heart of rats decapitated 24 hours after acute MPH administration. It has been known that SOD activity has an important role in biological systems due to its ability to transform the superoxide anion in hydrogen peroxide, which in turn is reduced by CAT and/or GPx [46, 47]. Our results suggest that the alterations observed in these antioxidant enzymes were not enough to prevent the changes in the DCF formed, suggesting that the reactive species responsible by changes in the levels of DCF and damage markers (lipids and proteins) were not neutralized by these antioxidant systems.

In chronic administration, we observed that DCF formed was increased in heart of rats at 2 and 24 hours after the last administration of MPH. SOD activity was not altered at 2 hours, but increased at 24 hours after MPH administration. CAT activity and SOD/CAT ratio were not altered, while GPx activity was decreased by chronic MPH administration at any time evaluated. An increase in TRAP in the heart of rats at 2 and 24 fours after chronic administration of MPH was observed. At 2 and 24 hours after chronic administration of MPH, we observed that the levels of TBARS and TNB, as well as carbonyl content were not altered in heart of rats. Since the results from chronic treatment were similar in both at 2 and 24 h, we suggest that the effects of MPH in heart of rats are independent of time of administration. Despite the

increase in production of reactive species observed, which could be explained by the decrease in GPx activity and the lack of the effect of MPH on damage to biomolecules (lipids and proteins), an adaptation mechanisms of the body chronically exposed to MPH could have occurred as a consequence.

Nitric oxide, synthesized by nitric oxide synthase present in endothelium of blood vessels, can play important physiological roles in the regulation of cardiovascular function, as in the modulation of blood flow, platelet aggregation and inflammation [48]. On the other hand, nitric oxide can also be deleterious for the heart, since it is a free radical of nitrogen [49]. In this context, nitric oxide production in heart may have a profound impact on cardiac function through both vascular-independent and vacular-dependent effects. Coronary vessel tone, thrombogenicity, inflammatory process and angiogenesis are some effects regulated by mechanisms vascular-independent. Direct effects of nitric oxide on myocardical contractility and diastolic function are mediated by changes in the excitation-contraction coupling, presynaptic and postsynaptic modulation of autonomic signaling, as well as changes in mitochondrial respiration [50, 51].

In the present study we demonstrated an increase in nitrite levels, the main metabolite of nitric oxide, in heart of rats at 2 and 24 hours after a single administration of MPH. On the other hand, heart of rats that received chronic MPH administration we did not observed changes in nitrite levels in any time tested after the last administration of MPH. Since nitric oxide can react with thiol groups (an important signaling pathway) and/or superoxide anion to form peroxynitrite that besides contributing to decreased relaxation mediated by nitric oxide [52] also is a potent oxidant that causes damage to biomolecules [53-55], we suggest, that acute administration of MPH provoked not only an increase in production of nitric oxide in

heart of rats but also an increase in its spontaneous reaction with superoxide anion and a decrease in its reaction with thiol groups. This hypothesis could explain the increase in damage to biomolecules (TBARS levels and carbonyl groups) and thiol groups (TNB levels) observed in our study. On the other hand, in chronic administration of MPH, the nitric oxide synthesis seems not to be changed, as well as its reaction with superoxide anion and thiol groups, suggesting an adaptative effect by chronic treatment with MPH.

Taken together our findings suggest that the effects of MPH on oxidative/nitrative stress in heart depend on the duration of treatment, since we demonstrated that acute administration promoted more changes in the parameters evaluated than chronic administration of MPH in heart of rats. These data are consistent with previous studies that showed that long-term exposure can promote tissue adaptation to avoid changes that may be harmful to the body [56, 57]. Furthermore, studies show that the more negative effects on the heart including acute dilated cardiomyopathy in young adults [58, 59].

Experimental models are very useful in the investigation of pharmacological and toxicological mechanisms of a drug to ensure the safety of users [60]. But so far, to our knowledge, there is no experimental study investigating the possible involvement of free radicals in cardiomyopathies promoted by treatment with MPH. In this context, changes such as elevations of heart rate and blood pressure are often described, while less is known about the rare etiology of cardiovascular events or long-term sequelae by MPH treatment [11]. On the other hand, little is known about the mechanisms involved in negative effects promoted by MPH on the heart. Some studies report that changes in the cholinergic system [61, 62] and oxidative stress [42-44] could be involved. These data are consistent with a study recently conducted

by us that demonstrated that rats treated with MPH presented an increase in butyrylcholinesterase activity in blood, which is considered a risk factor for cardiovascular disease and can be altered by oxidative stress [42]. However, the presence of cardiovascular abnormalities in animals after MPH treatment has been controversial [63, 64].

In summary, this work presents a new approach on this subject, from the evidence, at least in part, the involvement of oxidative stress in cardiovascular effects associated with the use of MPH. However, more studies are needed to consolidate these findings and to understand more fully the mechanisms of redox system and other mechanisms involved in negative effects on the heart induced by treatment with MPH.

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References

1. Kirby, K., Rutman, L.E. and Bernstein, H. (2002). Attention-deficit/hiperactivity disorder: a therapeutic update. Curr. Opin. Pediatr. 14:236-246.
2. Witthöft, J., Koglin, U. and Petermann, F. (2010). Comorbidity of aggressive behavior and ADHD [in German]. Kindh Entwickl 19:218-227.
3. Zuvekas, S.H., Vitiello, B. and Norquist, G.S. (2006). Recent trends in stimulant medication use among U.S. children. Am. J. Psychiatric 163:579-585.
4. Preen, D.B., Calver, J., Sanfilippo, F.M., Bulsara, M. and Holman, C.D. (2007). Patterns of psychostimulant prescribing to children with ADHD in Western Australia: variations in age, gender, medication type and dose prescribed. Aust. N. Z. J. Public. Health. 31:120-126.
5. Winterstein, A.G., Gerhard, T., Shuster, J., Zito, J., Johnson, M., Liu, H. and Saidi, A. (2008). Utilization of pharmacologic treatment in youths with attention deficit/hyperactivity disorder in Medicaid database. Ann. Pharmacother. 42:24-31.
6. Habel, L.A., Cooper, W.O., Sox, C.M., Chan, K.A., Fireman, B.H., Arbogast, P.G., Cheetham, T.C., Quinn, V.P., Dublin, S., Boudreau, D.M., Andrade, S.E., Pawloski, P.A., Raebel, M.A., Smith, D.H., Achacoso, N., Uratsu, C., Go, A.S., Sidney, S., Nguyen-Huynh, M.N., Ray, W.A. and Selby, J.V. (2011). ADHD Medications and Risk os Serious Cardiovascular Events in Young and Middle-aged Adults. JAMA 306:2673-2683.
7. Zito, J.M., Safer, D.J., dos Reis, S., Gardner, J.F., Boles, M. and Lynch, F. (2000). Trends in the prescribing of psychotropic medications to preschoolers. JAMA 283:1025-1030.

8. Rowland, S.A., Lesesne, C.A. and Abramowitz, A.J. (2002). The epidemiology of attention-deficit/hyperactivity disorder (ADHD): a public health view. Ment. Retard. Dev. Disabil. Res. Rev. 8:162-170.
9. Cooper, W.O., Habel, L.A., Sox, C.M., Chan, K.A., Arbogast, P.G., Cheetham, T.C., Murray, K.T., Quinn, V.P., Stein, C.M., Callahan, S.T., Fireman, B.H., Fish, F.A., Kirshner, H.S., O'Duffy, A., Connell, F.A. and Ray, W.A. (2011). ADHD drugs and serious cardiovascular events in children and young adults. N. Engl. J. Med. 365:1896-1904.
10. Figueredo, V.M. (2011). Chemical cardiomyopathies: the negative effects of medications and nonprescribed drugs on the heart. Am. J. Med. 124:480-488.
11. Hammerness, P.G., Surman, C.B. and Chilton, A. (2011). Adult attention-deficit/hyperactivity disorder treatment and cardiovascular implications. Curr. Psychiatry Rep. 13:357-363.
12. Martinez-Raga, J., Knecht, C., Szerman, N. and Martinez, M.I. (2013). Risk of serious cardiovascular problems with medications for attention-deficit hyperactivity disorder. CNS Drugs 27:15-30.
13. Stiefel, G. and Besag, F.M. (2010). Cardiovascular effects of methylphenidate, amphetamines and atomoxetine in the treatment of attention-deficit hyperactivity disorder. Drug Saf. 33:821-842.
14. Lewington, S., Clarke, R., Qizilbash, N., Peto, R. and Collins, R. (2002). Prospective Studies Collaboration. Age-specific relevance of usual blood pressure to vascular mortality: a meta-analysis of individual data for one million adults in 61 prospective studies. Lancet. 360:1903-1913.

15. Valko, M., Rhodes, C.J., Moncol, J., Izakovic, M. and Mazur, M. (2006). Free radicals, metals and antioxidants in oxidative stress-induced cancer. Chem Biol Interact 160:1-40.
16. Halliwell, B. and Gutteridge, J.M.C. (2007). Cellular responses to oxidative stress: Adaptation, damage, repair, senescence and death. New York: Oxford University Press.
17. Volkow, N.D., Wang, G.J., Fowler, J.S., Gatley, S.J., Logan, J., Ding, Y.S., Hitzemann, R. and Pappas, N. (1998). Dopamine transporter occupancies in the human brain induced by therapeutic doses of oral methylphenidate. Am. J. Psychiatry 155:1325-1331.
18. Gerasimov, M.R., Francesch, M., Volkow, N.D., Gifford, A., Gatley, S.J., Marsteller, D., Molina, P.E. and Dewey, S.L. (2000). Comparison between intraperitoneal and oral methylphenidate administration: A microdialysis and locomotor activity study. J. Pharmaco.l Exp. Ther. 295:51-57.
19. Leonard, B.E., McCartan, D., White, J. and King, D.J. (2004). Methylphenidate: review of its neuropharmacological, neuropsychological and adverse clinical effects. Hum. Psychopharmacol. Clin. Exp. 19:151-180.
20. Rice, D. and Barone, S.Jr. (2000). Critical periods of vulnerability for the developing nervous system: evidence from humans and animal models. Environ. Health Perspect. 108:511-533.
21. Andreazza, A.C., Frey, B.N., Valvassori, S.S., Zanotto, C., Gomes, K.M., Comim, C.M., Cassini, C., Stertz, L., Ribeiro, L.C., Quevedo, J., Kapczinski, F., Berk, M. and Gonçalves, C.A. (2007). DNA damage in rats after treatment with methylphenidate. Prog. Neuropsychopharmacol. Biol. Psychiatry 31:1282-1288.

22. LeBel, C.P., Ischiropoulos, H., Bondy, S.C. (1992). Evaluation of the probe 2',7'- dichlorofluorescin as an indicator of reactive oxygen species formation and oxidative stress. Chem. Res. Toxicol. 5:227–231.
23. Ignarro, L.J., Fukuto, J.M., Griscavage, J.M., Rogers, N.E. and Byrns, R.E. (1993). Oxidation of nitric oxide in aqueous solution to nitrite but not nitrate: comparison with enzymatically formed nitric oxide from L-arginine. Proc. Natl. Acad. Sci. USA 90:8103-8107.
24. Green, L.C., Wagner, D.A., Glogowski, J., Skipper, P.L., Wishnok, J.S. and Tannenbaum, S.R. (1982). Analysis of nitrate, nitrite, and [15N]nitrate in biological fluids. Anal. Biochem. 126:131-138.
25. Marklund, S.L. (1985). Pyrogallol autoxidation. In: Greenwald RA, editor. Handbook for oxygen radical research. Boca Raton: CRC Press. pp 243–247.
26. Aebi, H. (1984). Catalase in vitro. Methods Enzymol. 105:121–126.
27. Wendel, A. (1981). Glutathione peroxidase. Methods Enzymol. 77:325–333.
28. Lissi, E., Salim-Hanna, M., Pascual, C. and del Castillo, M.D. (1995). Evaluation of total antioxidant potential (TRAP) and total antioxidant reactivity from luminol enhanced chemiluminescence measurements. Free. Radic. Biol. Med. 18:153–158.
29. Evelson, P., Travacio, M., Repetto, M., Escobar, J., Llesuy, S. and Lissi, E.A. (2001). Evaluation of total reactive antioxidant potential (TRAP) of tissue homogenates and their cytosols. Arch. Biochem. Biophys. 388:261–266.
30. Ohkawa, H., Ohishi, N. and Yagi, K. (1979). Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal. Biochem. 95:351–358.

31. Stadtman, E.R. and Levine, R.L. (2003). Free radical-mediated oxidation of free amino acids and amino acid residues in proteins. Amino Acids 25:207–218.
32. Reznick, A.Z. and Packer, L. (1994). Oxidative damage to proteins: Spectrophotometric method for carbonyl assay. Methods Enzymol. 233:357–363.
33. Aksenov, M.Y. and Markesbery, W.R. (2001). Changes in thiol content and expression of glutathione redox system genes in the hippocampus and cerebellum in Alzheimer's disease. Neurosci. Lett. 302:141–145.
34. Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951). Protein measurement with the Folin phenol reagent. J. Biol. Chem. 193:265–275.
35. Kolling, J., Scherer, E.B., da Cunha, A.A., da Cunha, M.J. and Wyse, A.T. (2011). Homocysteine induces oxidative-nitrative stress in heart of rats: prevention by folic acid. Cardiovasc. Toxicol. 11:67:73.
36. Kelleher, K.J., McInerny, T.K., Gardner, W.P., Childs, G.E. and Wasserman, R.C. (2000). Increasing identification of psychosocial problems. Pediatrics 105:1313-1321.
37. Mandell, D.S., Thompson, W.W., Weintraub, E.S., Destefano, F. and Blank, M.B. (2005). Trends in diagnosis rates for autism and ADHD at hospital discharge in the context of other psychiatric diagnoses. Psychiatric. Serv. 56:56-62.
38. Polanczyk, G., de Lima, M.S., Horta, B.L., Biederman, J. and Rohde, L.A. (2007). The worldwide prevalence of ADHD: a systematic review and metaregression. Am. J. Psychiatric. 164:942-948.

39. Hodgkins, P., Sasané, R. and Meijer, W.M. (2011). Pharmacologic treatment of attention-deficit/hyperactivity in children: incidende, prevalence, and treatment patterns in the Netherlands. Clin. Ther. 133:188-203.
40. Chase, T.D., Brown, R.E., Carrey, N., Wilkinson M. (2003). Daily methylphenidate administration attenuates c-fos expression in the striatum of prepubertal rats. Neuroreport 15:769-772.
41. Schmitz, F., Scherer, E.B., Machado, F.R., da Cunha, A.A., Tagliari, B., Netto, C.A. and Wyse, A.T. (2012a). Methylphenidate induces lipid and protein damage in prefrontal cortex, but not in cerebellum, striatum and hippocampus of juvenile rats. Metab. Brain. Dis. 27:605-612.
42. Schmitz, F., Scherer, E.B., da Cunha, M.J., da Cunha, A.A., Lima, D.D., Delwing, D., Netto, C.A. and Wyse AT. (2012b). Chronic methylphenidate administration alters antioxidant defenses and butyrylcholinesterase activity in blood of juvenile rats. Mol. Cell. Biochem. 361:281-288.
43. Lemieux, H., Bulteau, A.L., Friguet, B., Tardif, J.C. and Blier, P.U. (2010). Dietary fatty acids and oxidative stress in the heart. Mitochondrion 11:97-103.
44. Pillai, V.B., Sundaresan, N.R., Jeevanandam, V. and Gupta, M.P. (2010). Mitochondrial SIRT3 and heart disease. Cardiovasc. Res. 88:250-256.
45. Stehr, C.B., Mellado, R., Ocaranza, M.P., Carvajal, C.A., Mosso, L., Becerra, E., Solis, M., García, L., Lavandero, S., Jalil, J. and Fardella, C.E. (2010). Increased levels of oxidative stress, subclinical inflammation, and myocardical fibrosis markers in primary aldosteronism patients. J. Hypertens. 28:2120:2126.

46. Matés, J.M., Pérez-Gómez, C. and Núñez de Castro I. (1999). Antioxidant enzymes and human diseases. Clin. Biochem. 32:595-603.

47. Gutteridge, J.M.C. (2001). Free Radicals in Biology and Medicine. 4th ed. Oxford University Press Inc, New York, pp 851.

48. Becker, J.S., Adler, A., Schneeberger, A., Huang, H., Wang, Z., Walsh, E., Koller, A. and Hintze, T.H. (2005). Hiperhomocysteinemia, a cardiac metabolic disease role of nitric oxide and the p22*phox* subunit of NADPH oxidase. Circulation 111:2112-2118.

49. Tuteja, N., Chandra, M., Tuteja, R. and Misra, M.K. (2004). Nitric oxide as a unique bioactive signaling messenger in physiology and pathophysiology. J. Biomed. Biotechnol. 4:227-237.

50. Massion, P., Feron, O., Dessy, C. and Balligand, J. (2003). Nitric oxide and cardiac function: ten years after, and continuing. Circ. Res. 93:388-398.

51. Casadei, B. (2006). The experimental role of neuronal nitric oxide synthase in the regulation of myocardical function. Exp. Physiol. 91:943-955.

52. Beckman, J.S. and Koppenol, W.H. (1996). Nitric oxide, superoxide, and peroxynitrite: The good, the bad, and ugly. Am. J. Physiol. 271:1424-1437.

53. Sarkar, A. and Bhaduri, A. (2001). Black tea is a powerful chemopreventor of reactive oxygen and nitrogen species: comparison with its individual catechin constituents and green tea. Biochem. Biophys. Res. Commun. 284:173-178.

54. Bowler, R.P. and Crapo, J.D. (2002). Oxidative stress in airways: is there a role for extracellular superoxide dismutase? Am. J. Respir. Crit. Care Med. 166:38-43.

55. Radi, R., Cassiana, A., Hodara, R., Quijano, C. and Castro, L. (2002). Peroxynitrite reactions and formation in mitochondria. Free Radic. Biol. Med. 33:1451-1464.

56. Martins, M.R., Reinke, A., Petronilho, F.C., Gomes, K.M., Dal-Pizzol, F. and Quevedo, J. (2006). Methylphenidate treatment induces oxidative stress in young rat brain. Brain. Res. 1078:189-197.

57. Gomes, K.M., Inácio, C.G., Valvassori, S.S., Réus, G.Z., Boeck, C.R., Dal-Pizzol, F. and Quevedo, J. (2009). Superoxide production after acute and chronic treatment with methylphenidate in young and adult rats. Neurosci. Lett. 465:95-98.

58. Tollofsrud, C. and Hoel, T. (2006). A young man with acute dilated cardiomyopathy. Todsskr. Nor. Laegeforen. 126:1338-1339.

59. Nymark, T.B., Hovland, A., Bjornstad, H. and Nielsen, E.W. (2008). A young man with acute dilated cardiomyopathy associated with methylphenidate. Vasc. Health. Risk. Manag. 4:477-479.

60. Fukami, G., Hashimoto, K., Koike, K., Okamura, N., Shimizu, E., Iyo, M. (2004). Effect of antioxidant N-acetyl-cysteine on behavioral changes and neurotoxicity in rats after administration of methamphetamine. Brain Res 1016:90-95.

61. Crippa, G.E., Peres-Polon, V.L., Kuboyama, R.H. and Correa, F.M. (1999). Cardiovascular response to the injection of acetylcholine into the anterior region of medial prefrontal cortex of un anesthetized rats. Cereb. Cortex. 9:362-365.

62. Darvesh, S., Hopkins, D.A. and Geula, C. (2003). Neurobiology of butyrylcholinesterase. Nat. Ver. Neurosci. 17:131-138.

63. Take, G., Bahcelioglu, M., Oktem, H., Tunc, E., Gözil, R., Erdogan, D., Calguner, E.,Helvacioglu F., Giray, S.G. and Elmas, C. (2008). Dose-dependent immunohistochemical and ultrastructural changes after oral methylphenidate administration in rat heart tissue. Anat. Histol. Embryol. 37:303-308.

64. Witt, K.L., Malarkey, D.E., Hobbs, C.A., Davis, J.P., Kissling, G.E., Caspary, W.,Travlos, G. and Recio, L. (2010). No increases in biomarkers of genetic damage or pathological changes in heart and brain tissues in male rats administered methylphenidate hydrochloride (Ritalin) for 28 Days. Environ. Mol. Mutagen. 51:80-88.

Legends to Figures

Figure 1. Effect of acute and chronic methylphenidate administration on reactive species levels (DCF formed) (A) and nitrite levels (B) in the heart of rats decapitated at 2 or 24 hours after injection of methylphenidate. Results are expressed as mean ± S.D. (n = 6 - 10 per group). Data were analyzed by one-way ANOVA followed by Tukey HSD Post-Hoc Tests. Different from control, *** $p < 0.001$ and ** $p < 0.01$.

Figure 2. Effect of acute and chronic methylphenidate administration on SOD activity (A), CAT activity (B), SOD/CAT ratio (C) and GPx activity (D) in the heart of rats decapitated at 2 or 24 hours after injection of methylphenidate. Results are expressed as mean ± S.D. (n = 5 - 10 per group). Data were analyzed by one-way ANOVA followed by Tukey HSD Post-Hoc Tests. Different from control, ** $p < 0.01$ and * $p < 0.05$.

Figure 3. Effect of acute and chronic methylphenidate administration on TRAP in the heart of rats decapitated at 2 or 24 hours after injection of methylphenidate. Results are expressed as mean ± S.D. (n = 5 - 10 per group). Data were analyzed by one-way ANOVA followed by Tukey HSD Post-Hoc Tests. Different from control, *** $p < 0.001$.

Figure 4. Effect of acute and chronic methylphenidate administration on TBARS levels (A), carbonyl content (B) and TNB levels (C) in the heart of rats decapitated at 2 or 24 hours after injection of methylphenidate. Results are expressed as mean ± S.D. (n = 5 - 11 per group). Data were analyzed by one-way ANOVA followed by

Tukey HSD Post-Hoc Tests. Different from control, *** $p < 0.001$, ** $p < 0.01$ and * $p < 0.05$.

Fig. 1

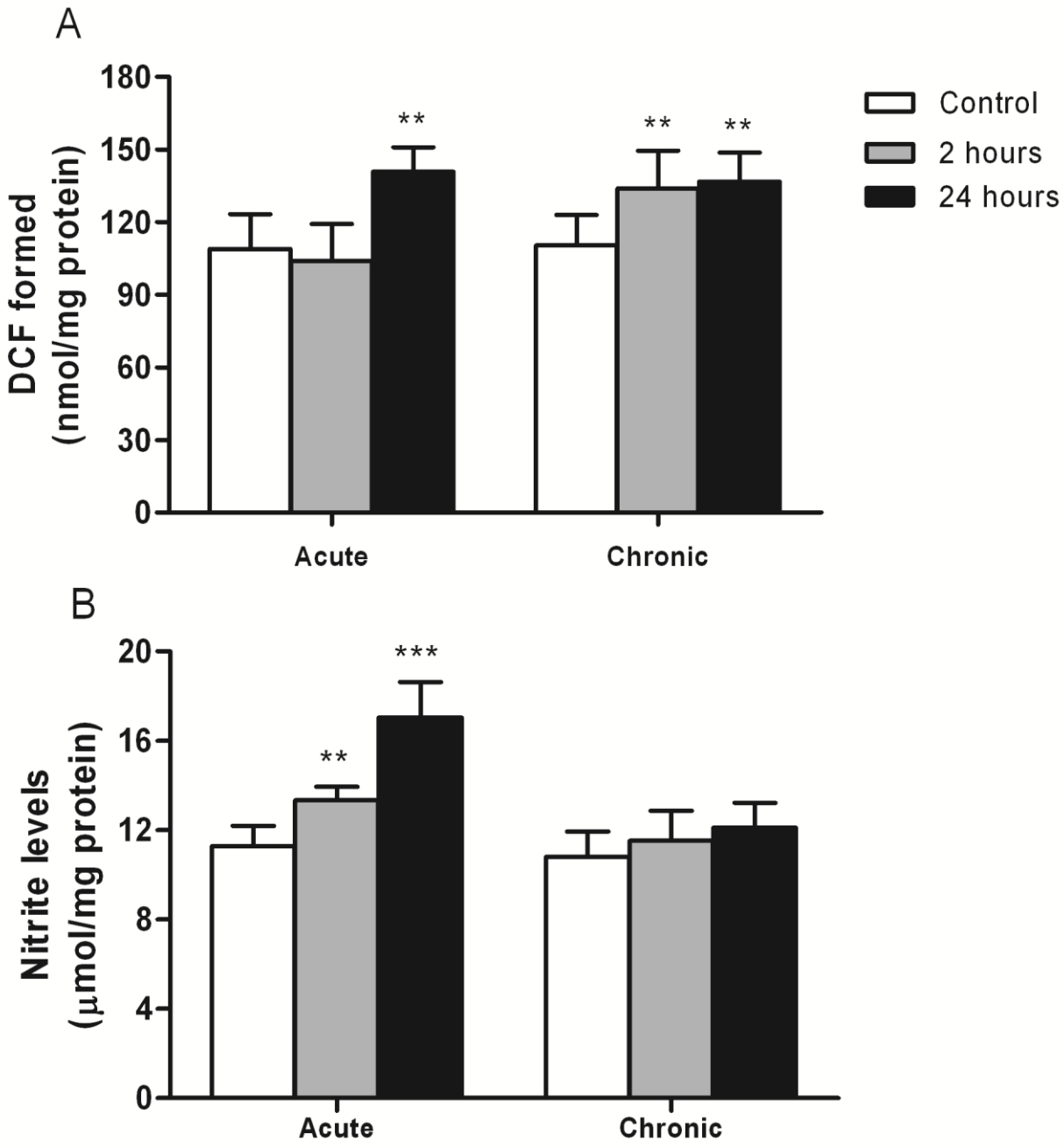

A
DCF formed
(nmol/mg protein)
180
150
120
90
60
30
0
**
**
**
Acute
Chronic
Control
2 hours
24 hours
B
Nitrite levels
(µmol/mg protein)
20
16
12
8
4
0
**
***
Acute
Chronic

Fig. 2

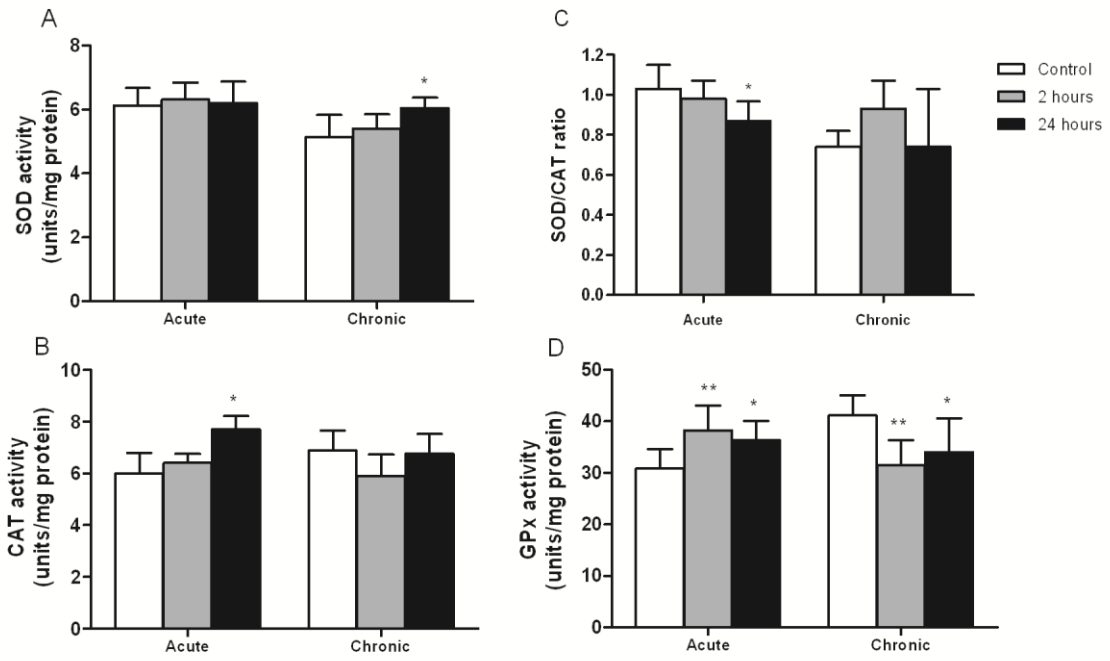

A
SOD activity (units/mg protein)
8
6
4
2
0
*
Acute
Chronic
C
SOD/CAT ratio
1.2
1.0
0.8
0.6
0.4
0.2
0.0
*
Acute
Chronic
Control
2 hours
24 hours
B
CAT activity (units/mg protein)
10
8
6
4
2
0
*
Acute
Chronic
D
GPx activity (units/mg protein)
50
40
30
20
10
0
**
*
**
*
Acute
Chronic

Fig. 3

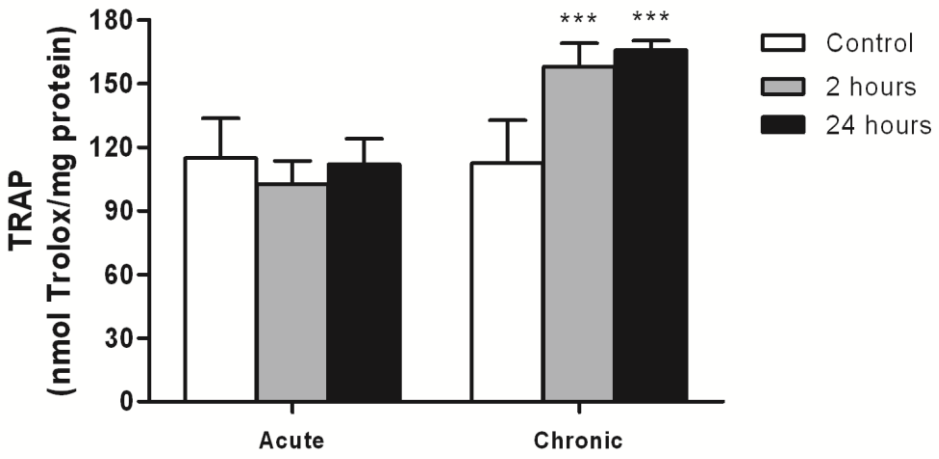
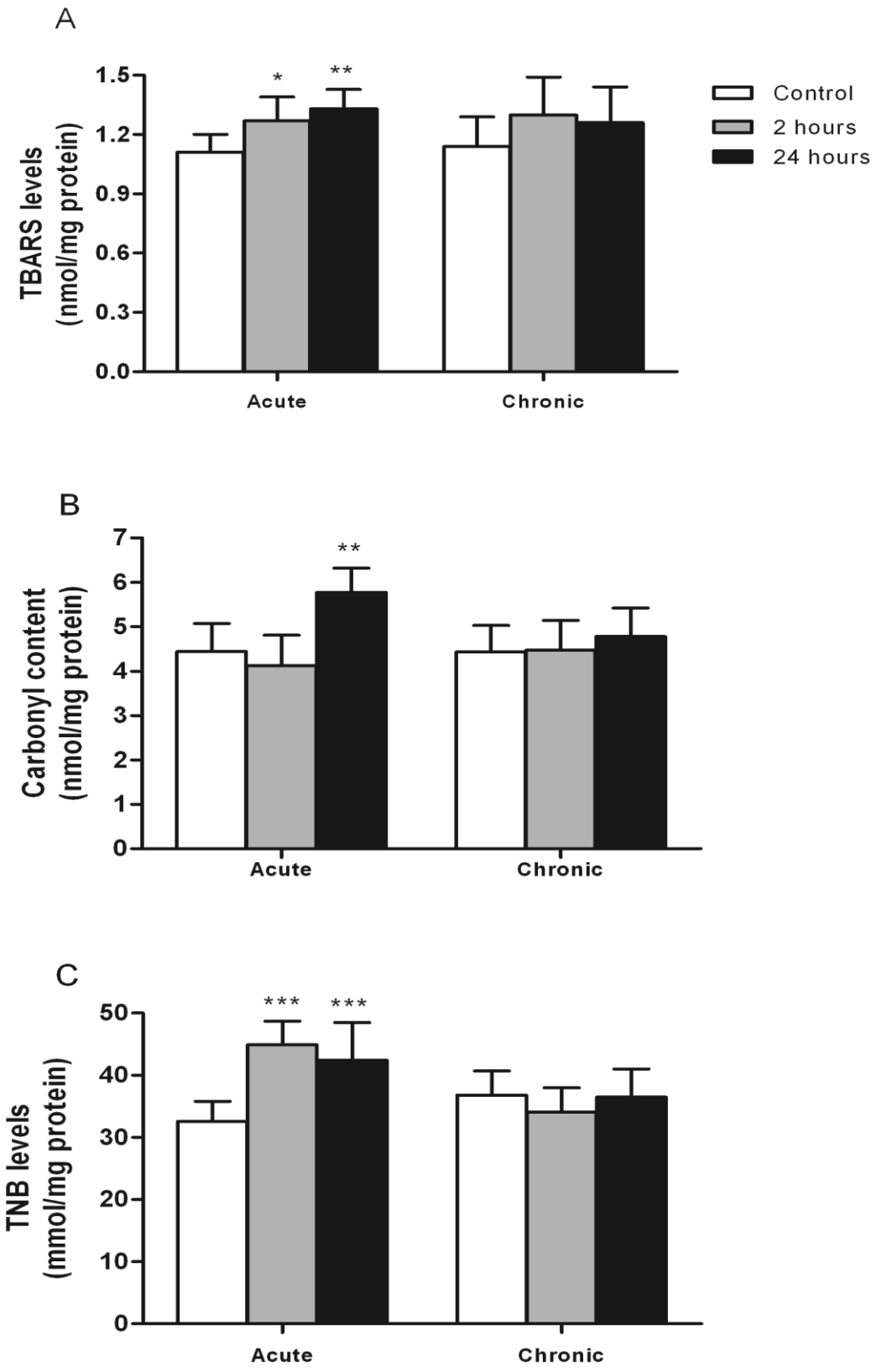

TRAP
(nmol Trolox/mg protein)
180
150
120
90
60
30
0
***
***
Acute
Chronic
Control
2 hours
24 hours

Fig. 4


A
TBARS levels (nmol/mg protein)
1.5
1.2
0.9
0.6
0.3
0.0
*
**
Acute
Chronic
Control
2 hours
24 hours
B
Carbonyl content (nmol/mg protein)
7
6
5
4
3
2
1
0
**
Acute
Chronic
C
TNB levels (mmol/mg protein)
50
40
30
20
10
0
***
***
Acute
Chronic

Perspectivas

1. Medir a frequência cardíaca e pressão sanguínea de animais submetidos à administração de metilfenidato;
2. Realizar análises morfológicas de coração e aorta de animais submetidos à administração de metilfenidato;
3. Determinar parâmetros de metabolismo energético em coração de animais submetidos à administração de metilfenidato, como a atividade das enzimas piruvato quinase, citrato sintase, citocromo c oxidase, bem como os níveis de ATP intracelular;
4. Investigar alterações na função cardíaca através da determinação de biomarcadores séricos em animais submetidos à administração de metilfenidato, como as subunidades da troponina, mioglobina, lactato desidrogenase e outros.