



BASIC SCIENCE ARTICLE

A randomized controlled laboratory study on the long-term effects of methylphenidate on cardiovascular function and structure in rhesus monkeys

James D. Wilkinson¹, Ralph Callicott², William F. Salminen³, Satinder K. Sandhu⁴, James Greenhaw⁵, Angel Paredes⁶, Kelly Davis⁷, Yvonne Jones⁶, Merle G. Paule⁸, William Slikker Jr.⁹, Paolo G. Rusconi⁴, Jason Czachor¹⁰, Amy Bodien¹⁰, Joslyn A. Westphal¹⁰, Danielle D. Dauphin¹¹ and Steven E. Lipshultz¹²

BACKGROUND: Whether long-term methylphenidate (MPH) results in any changes in cardiovascular function or structure can only be properly addressed through a randomized trial using an animal model which permits elevated dosing over an extended period of time.

METHODS: We studied 28 male rhesus monkeys (*Macaca mulatta*) approximately 7 years of age that had been randomly assigned to one of three MPH dosages: vehicle control (0 mg/kg, b.i.d., $n = 9$), low dose (2.5 mg/kg, b.i.d., $n = 9$), or high dose (12.5 mg/kg, b.i.d., $n = 10$). Dosage groups were compared on serum cardiovascular and inflammatory biomarkers, electrocardiograms (ECGs), echocardiograms, myocardial biopsies, and clinical pathology parameters following 5 years of uninterrupted dosing.

RESULTS: With the exception of serum myoglobin, there were no statistical differences or apparent dose-response trends in clinical pathology, cardiac inflammatory biomarkers, ECGs, echocardiograms, or myocardial biopsies. The high-dose MPH group had a lower serum myoglobin concentration (979 ng/mL) than either the low-dose group (1882 ng/mL) or the control group (2182 ng/mL). The dose response was inversely proportional to dosage ($P = .0006$).

CONCLUSIONS: Although the findings cannot be directly generalized to humans, chronic MPH exposure is unlikely to be associated with increased cardiovascular risk in healthy children.

Pediatric Research (2019) 85:398–404; <https://doi.org/10.1038/s41390-018-0256-9>

INTRODUCTION

Methylphenidate (MPH) is a mild central nervous system stimulant used to treat attention-deficit/hyperactivity disorder (ADHD) and related conditions. Currently, more than 1.8 million children are prescribed MPH in the United States each year.¹ Available in the United States as a prescription drug since the 1950s, MPH came into widespread use in children and adolescents in the 1970s, when it was recognized that stimulants, such as MPH, could help control the symptoms of ADHD.

Case studies continue to speculate about a link between chronic MPH use and various cardiovascular abnormalities and conditions including cardiomyopathy and acute myocardial injury.^{2–5} Such studies are highly biased since the large number of subjects who do not manifest complications are not reported. Both the American Heart Association (AHA) and the American Academy of Pediatrics (AAP) specifically advised physicians to screen their patients for underlying cardiovascular problems using detailed child and family histories and thorough physical

examinations before prescribing MPH or any other stimulant drugs.^{6,7} Currently, there is no consensus on the value of an electrocardiogram (ECG) as part of the pre-prescribing screening. The AHA recommendations state that obtaining a screening ECG is reasonable but not mandatory before starting stimulant medications in children, whereas the AAP states “a recommendation to obtain routine ECGs for children receiving ADHD medications is not warranted.”⁸

In 2009, Gould et al.⁹ reported the results from a large case-control study which found that children who experienced sudden death were seven times more likely to be taking stimulants, including MPH for ADHD, compared to a control group who died in automobile accidents. However, three commentaries on the topic of MPH and cardiovascular risk from the same era came to different conclusions which are described here. One editorial recommended more selective and restricted use and urged caution in prescribing MPH and other stimulants for ADHD in response to the FDA’s (Food and Drug

¹Department of Pediatrics, Vanderbilt University School of Medicine, Nashville, TN 37232, USA; ²Animal Resource Center, University of Texas Southwestern Medical Center, Dallas, TX 75390, USA; ³Toxicology and Formulations, Pro Natural Brands, LLC, Victor, NY 14564, USA; ⁴Department of Pediatrics, University of Miami Miller School of Medicine, Miami, FL 33156, USA; ⁵Division of Systems Biology, National Center for Toxicological Research, US Food and Drug Administration, Jefferson, AR 72079, USA; ⁶Office of Scientific Coordination, National Center for Toxicological Research, US Food and Drug Administration, Jefferson, AR 72079, USA; ⁷Toxicologic Pathology Associates, Jefferson, AR 72079, USA; ⁸Division of Neurotoxicology, National Center for Toxicological Research, US Food and Drug Administration, Jefferson, AR 72079, USA; ⁹Office of the Center Director, National Center for Toxicological Research, US Food and Drug Administration, Jefferson, AR 72079, USA; ¹⁰Department of Pediatrics, Wayne State University School of Medicine and Children’s Hospital of Michigan, Detroit, MI 48201, USA; ¹¹Department of Neurology, University of Miami Miller School of Medicine, Miami, FL 33156, USA and ¹²Department of Pediatrics, University at Buffalo Jacobs School of Medicine and Biomedical Sciences, Oishei Children’s Hospital, Roswell Park Comprehensive Cancer Center, Buffalo, NY 14203, USA
Correspondence: Steven E. Lipshultz (slipshultz@upa.chob.edu)

Received: 18 June 2018 Accepted: 16 November 2018

Published online: 17 December 2018

Administration) "Black-Box" in 2006 warning about MPH use and increased cardiovascular risk.¹⁰ A second perspective in *JAMA*, referring to the Kuehn et al.¹¹ report, noted that a branch chief at the National Institute for Mental Health observed that for the first time an association has been shown between MPH and sudden death, although the methodology was described as "not perfect." The third commentary stated, "The risk for sudden death incurred by youth taking medication for the treatment of ADHD is probably comparable to what the risk is in youth participating in competitive athletic activities."¹² Although more recent studies generally have not supported an association between MPH use and serious cardiac events,^{13,14} others continue to express concerns about adverse cardiovascular effects of MPH use.^{15,16} Despite expert guidelines from the AAP and others,^{6–8,17} these recent reports suggest that there is not a consensus regarding whether the chronic use of MPH or other stimulants in children or adults increases the risk of serious cardiovascular events, including sudden death.

Two studies in rats and mice have shown abnormal and degenerative ultrastructural changes in the myocardium of animals exposed to MPH.^{18,19} However, another study in rats exposed to MPH found no histopathological changes in the heart or elevated serum biomarkers of cardiac injury.²⁰ None of the studies reported on LV gross morphology or function which we measured in this MPH-exposed rhesus monkey group.

The purpose of this study was to determine whether any of several cardiovascular endpoints were affected by chronic MPH exposure in rhesus monkeys. Reports from the same monkey colony used in this study showed no evidence of genetic toxicity in MPH doses using dosages comparable to those used in children.²¹ Also in the same colony, there was evidence of pubertal delay and abnormalities of glucose uptake in the MPH-treated groups. These findings or organ system-specific effects of chronic MPH exposure using this non-human primate model suggest that it is appropriate to study the effect of chronic MPH exposure on other organ systems such as the cardiovascular system.^{22,23}

We performed endomyocardial biopsies for histopathological studies to compare to results from the non-primate animal models referenced in the preceding paragraph. We also measured echocardiographic parameters and serum biomarker levels that are routinely used in studies of cardiotoxicity in cancer patients exposed to cardiotoxic chemotherapy regimens and human immunodeficiency virus-infected patients exposed to antiretrovirals, among others. The monkeys were continuously treated with MPH for 5 years at therapeutic and super-therapeutic doses using a three-arm randomized design starting at approximately 2 years of age, which corresponds to a developmental span from childhood to adulthood in humans.

MATERIALS AND METHODS

The study was approved by the US FDA, National Center for Toxicological Research (NCTR) Institutional Animal Care and Use Committee. All animals were cared for in accordance with the National Research Council's "Guide for the Care and Use of Laboratory Animals." All animal procedures were approved by the NCTR Institutional Animal Care and Use Committee and conducted in full accordance with the PHS Policy on the Humane Care and Use of Laboratory Animals.

The study protocol was approved by the Institutional Animal Care and Use Committee of the University of Miami, Miami, FL.

Experimental animals and habitat and care

Thirty male rhesus macaques (*Macaca mulatta*) were obtained from Alpha Genesis Inc. (Yemassee, SC, USA), were of Indian origin, and were natural habitat reared. Each was chronically exposed to MPH starting at approximately 2 years of age. The results of

previous genetic, behavioral, and developmental testing in these animals have been published.^{22,23} At the time of cardiovascular assessments, the animals were approximately 7 years old and weighed between 8.6 and 11.2 kg.

Animals were housed individually at room temperatures between 23 and 29 °C, with ambient humidity maintained between 35 and 75%, 10-to-15 air changes per hour, and fluorescent lighting provided on a 12-h on and 12-h off cycle, except during designated procedures. Filtered tap water was provided ad libitum. Feed consisted of High Protein Monkey Diet Jumbo (#5047, PMI Nutrition International, Richmond, IN, USA) daily, supplemented with children's vitamins and fresh fruit three times a week. The amount of feed offered was adjusted weekly to maintain a normal, healthy weight. As part of the environmental enrichment program, all animals underwent daily behavioral testing using banana-flavored reward pellets (Bioserv, Frenchtown, NJ, USA). Both the primate feed and reward pellets were lot-tested before use to confirm acceptable concentrations of dietary nutrients (e.g., fat, protein, and vitamins) and to ensure that contaminants (heavy metals, fumonisin, aflatoxin, and pesticides) were within acceptable concentrations.

Methylphenidate dosing and experimental design

Dextro-levo-methylphenidate hydrochloride (Mallinckrodt Chemicals, St. Louis, USA; MPH) was suspended in PRANGTM vehicle (LBS Biotechnology, UK), an oral rehydrator with electrolytes. The experimental design was a randomized laboratory study (one-way layout, animals nested within treatment conditions) in which animals were randomly assigned to one of three MPH treatment groups. Each of the three MPH dosages was given twice daily (b.i.d.): vehicle control (0 mg/kg, b.i.d., $n = 10$), low dose (2.5 mg/kg, b.i.d., $n = 10$), or high dose (12.5 mg/kg, b.i.d., $n = 10$). The two daily doses were separated by approximately 4 h, 5 days each week, followed by 2 days of no administration. The drug was administered orally at a volume of 0.5 mL/kg onto each animal's tongue and the animals were observed to ensure they swallowed the entire dose. The animals were treated for 5 years without interruption.

Based on analytically confirmed plasma levels, the 2.5 mg/kg twice-daily doses provided systemic exposure concentrations typically observed in children taking a therapeutic dose of MPH. The 12.5 mg/kg twice-daily dose represented systemic exposure concentrations that may be achieved in select patients or patients taking an overdose of MPH. Plasma concentrations in these animals were approximately 4–12 and 52–135 ng/mL at the 2.5 and 12.5 mg/kg b.i.d. doses, respectively.^{24,25}

Behavior testing

Five times each week (Monday through Friday), the animals underwent neurobehavioral testing after the morning dose. They were not tested on the weekends when they received no drug. This testing served two purposes: animal enrichment and assessment of the chronic effects of MPH exposure on neurobehavioral performance. The results of the neurobehavioral testing are reported elsewhere. While the neurobehavioral results are not germane to the current report, reporting the procedures here provides a better picture of the animal's daily routine.

Cardiovascular assessments and right ventricular biopsies

Because the right ventricular heart biopsies involved surgery, biopsies could only be obtained from two to four animals each day. The animals were randomly assigned to undergo surgery to minimize temporal effects. All biopsies were conducted on a dosing day, with only the morning dose administered to the animals undergoing surgery. The cardiovascular assessments involved the following steps:

Each animal was lightly anesthetized with an intramuscular (i.m.) injection of 10–20 mg/kg ketamine. Buprenorphine was also

given at 0.03 mg/kg i.m. for analgesia before transport to a mobile catheter laboratory.

After acquiring a 12-lead ECG, left ventricular function was assessed using two-dimensional echocardiography with Doppler and M-mode modalities. A board-certified pediatric cardiologist supervised the ECG recordings and acquired the echocardiograms. (A different board-certified pediatric cardiologist at the University of Miami, not involved in the study and blinded to the treatment condition, measured the ECGs and echocardiograms.)

Each animal was intubated and anesthetized with 1 to 5% isoflurane during the entire procedure. After positioning and preparing the animal for the procedure, a 5.5 Fr sheath was advanced using the percutaneous technique, from the right internal jugular vein to the right ventricle over a 0.025-in. J wire. A 5 Fr Cook biopsy forceps was used to obtain the biopsy. Hemostasis was obtained using hand pressure.

Blood samples were collected immediately before the heart biopsies from the biopsy catheter placed in the jugular vein. The samples later were analyzed for C-reactive protein, soluble tumor necrosis factor receptor 1, myoglobin, interleukin-6, interleukin-10, tumor necrosis factor- α , and cardiac troponin I.

The right ventricle was biopsied, the forceps removed from the sheath, and the tissue placed in 10% neutral-buffered formalin for light microscopy or 4% ice-cold glutaraldehyde for electron microscopy. Biopsies for light microscopy were fixed at room temperature, and those for electron microscopy were fixed in a refrigerator (~4–8 °C). After the biopsy, the forceps and sheath were removed, and hemostasis was again obtained using hand pressure. With only a few exceptions, five separate tissue sample biopsies from the right ventricle were obtained from each animal. Hemodynamic data was not measured during the cardiac catheterizations due to a lack of a hemodynamic console in the portable cardiac catheterization laboratory.

A post-procedure echocardiogram was obtained to document the absence of pericardial effusion. The animal was then extubated, closely monitored until fully recovered, and then returned to its home cage.

Light microscopy

Up to three biopsy samples were collected from each animal for light microscopy. To minimize sampling bias, the biopsies for light and electron microscopy were collected in random order from each animal, with a different random order for each animal.

After fixation in 10% neutral-buffered formalin at room temperature for at least 72 h, the samples were routinely processed and embedded in infiltrating medium for subsequent preparation of slides and hematoxylin and eosin (H&E) staining. After embedding, approximately 5- μ m-thick sequential sections were cut through the entire biopsy sample and placed on glass slides for H&E staining. The number of sections per biopsy ranged from 100 to 150, depending on the amount of the collected tissue. A board-certified veterinary pathologist, masked to treatment assignment, evaluated all sections from each biopsy. To reduce sampling bias, all of the sequential sections from each sample biopsy specimen were examined.

Electron microscopy

Because light microscopy might not reveal slight changes in organelles in response to treatment, biopsies from each animal were also examined using transmission electron microscopy. Up to two biopsies per animal were collected for electron microscopy analysis. After fixation of the tissues in 4% glutaraldehyde at 4–8 °C overnight, the tissues were stained in a 1% osmium tetroxide in 0.2 M Sorenson's phosphate buffer, pH 7.4, for 1 h. Once stained, the tissues were dehydrated in an increasing concentration of ethanol washes to 100%. Once dehydrated in ethanol, the tissues were transferred into propylene oxide in order to completely dehydrate the tissues. Infiltration with resin was

accomplished by infiltration through first a 1:2 mixture of Epon to propylene oxide, followed by a 2:1 mixture of Epon to propylene oxide and finally into 100% Epon. After infiltration, the resin was polymerized in a 60 °C oven overnight. Polymerized tissues were then sectioned using a Leica UC-6 ultramicrotome and the sections transferred to 200 mesh copper electron microscopy grids. The grids were then prepared for electron microscopy by post-staining them in lead citrate and uranyl acetate. Imaging was conducted using a JEOL JEM-2100 electron microscope operating at 80 keV for contrast. Images were recorded using either the Gatan Orius fast scan charged-coupled device camera or the Gatan 4k \times 4k ultrascan camera. A board-certified veterinary pathologist, masked to treatment, supervised the electron microscopy scanning and photography of representative sections.

Statistical methods

The basic design was a one-way completely randomized experiment (i.e., one independent factor at three levels). The general statistical approach for all outcomes, both continuous and discrete, was to provide dosage group-specific descriptive statistics (i.e., means, standard deviations, and proportions) and estimate exact *P* values associated with overall group differences using permutation tests. Permutation tests were chosen over parametric analysis of variance tests since they are exact, non-dependent on distributional assumptions (i.e., non-parametric), and easily extend to both continuous and discrete outcomes (i.e., means and proportions). Since multiple univariate testing was performed for each dependent effect, the test-wise Type I error rate was conservatively set at 0.01. In the event of a statistically significant overall dosage effect, the data were partitioned in order to calculate all possible group-specific pairwise permutation tests. Missing values other than those resulting from animal deaths (e.g., equipment failure) are noted in the tables.

Statistical calculations and graphics were performed using SAS (version 14.1) and SAS-JMP (version Pro 12) statistical software (SAS Institute, Cary, NC, USA). Exact *P* values from permutation tests were calculated using StatXact-5 (version, 11.1, Cytel Software Corporation, Cambridge, MA, USA).

RESULTS

At the end of the study, nine animals remained in the control group, nine in the low-dose group, and 10 in the high-dose group. One animal in the control group died after cardiac biopsy, which was attributed to the biopsy procedure. An animal in the low-dose group died before cardiac evaluation, but its cause of death could not be determined. Since it was in the low-dose group, the animal's decline in health and eventual death did not appear to be related to the MPH treatment.

The results for ECG and echocardiographic measurements, biomarkers of general inflammation, cardiovascular biomarkers, biomarkers of endothelial adhesion, fibrosis, and atherosclerosis, and lesions detected by light and electron microscopy are given in Tables 1–6, respectively. The only statistically significant difference between dosage groups was seen in the serum myoglobin concentration ($P = 0.0006$; Table 2). Figure 1 graphically presents the data by dosage group. The serum myoglobin decreased proportionally to the MPH dosage level. From a pairwise comparison perspective, the only statistically significant effect was seen at the 12.5 mg/kg b.i.d. dosage when compared to both the zero dosage ($P = 0.0025$) and 2.5 mg/kg b.i.d. dosage ($P = 0.0081$). No treatment-related clinical pathology changes were observed at any time during the study.

DISCUSSION

After comprehensive cardiovascular assessments in three MPH dosage groups in this non-human primate model, we found only

Table 1. Electrocardiographic outcomes of 28 rhesus macaque monkeys after receiving methylphenidate for 5 years, by dosage group

Outcome ^a	Methylphenidate dosage (mg/kg, b.i.d.)			P value ^a
	Control group (0), n = 9	Low-dose group (2.5), n = 9	High-dose group (12.5), n = 10	
Heart rate, mean (SD) (beats/min)	136 (11.2)	126 (5.4)	132 (9.4)	0.62
QT interval, mean (SD) (ms)	271 (30.6)	290 ^b (16.0)	281 ^c (28.5)	0.36
QTc interval, mean (SD) (ms)	400 (56.1)	418 ^b (27.2)	409 ^d (20.8)	0.69
QRS axis, mean (SD) (degrees)	64.3 (33.7)	29.0 (94.1)	84.3 (25.4)	0.12
QRS duration, mean (SD) (ms)	46.7 ^e (11.0)	51.7 (16.3)	47.2 (9.0)	0.68

^aType I error rate (α) = 0.01

^bAtrioventricular block, Q wave width <0.035, ST depression, non-specific ST and T wave changes, and ST elevation were negative for all animals. Probability values for statistical differences in means between dosage groups were calculated with exact permutation tests

^cn = 8

^dn = 9

^en = 6

Table 2. Echocardiographic outcomes of 28 rhesus macaque monkeys after receiving methylphenidate for 5 years, by dosage group

Outcome	Methylphenidate dosage (mg/kg, b.i.d.)			P value ^a
	Control group (0), n = 9 ^b	Low-dose group (2.5), n = 9	High-dose group (12.5), n = 10	
LV ED dimension, mean (SD) (mm)	2.5 (0.130)	2.48 (0.396)	2.44 (0.291)	0.92
LV ES dimension, mean (SD) (mm)	1.64 (0.151)	1.65 (0.313)	1.64 (0.293)	0.99
LV fractional shortening, mean (SD) (%)	34.5 (5.5)	33.4 (6.5)	33.0 (9.3)	0.91
ED septal wall thickness, mean (SD) (mm)	0.461 (0.029)	0.411 (0.035)	0.422 (0.034)	0.13
ES septal wall thickness, mean (SD) (mm)	0.541 (0.056)	0.488 (0.023)	0.524 (0.041)	0.04
LV ED posterior wall thickness, mean (SD) (mm)	0.445 (0.065)	0.428 (0.063)	0.466 (0.081)	0.50
LV ES posterior wall thickness, mean (SD) (mm)	0.532 (0.045)	0.530 (0.075)	0.545 (0.083)	0.88
LV mass, mean (SD) (g)	20.7 (4.7)	19.5 (4.8)	21.1 (5.0)	0.75

Probability values for statistical differences in means between dosage groups were calculated via exact permutation test

LV left ventricle, ED end diastolic, ES end systolic

^aType I error rate (α) = 0.01

^bOne monkey in the control group was excluded because it showed evidence of hypertrophic cardiomyopathy

Table 3. Concentrations of biomarkers for generalized inflammation and cardiomyocyte injury in 28 rhesus macaque monkeys after receiving methylphenidate for 5 years, by dosage group

Serum biomarker	Methylphenidate dosage (mg/kg, b.i.d.)			P value ^a
	Control group (0), n = 9	Low-dose group (2.5), n = 9	High-dose group (12.5), n = 10	
hsCRP, mean (SD) (mg/dL)	0.92 (0.52)	1.73 (1.93)	1.97 (1.03)	0.20
Soluble TNF R1, mean (SD) (U/mL)	1.36 (0.37)	1.25 (0.30)	1.21 (0.32)	0.60
Myoglobin, mean (SD) (ng/mL)	2182 (974)	1882 (798)	979 (142)	0.006
IL-6, % detectable	44.4	33.3	60.0	0.57
IL-10, % detectable	22.2	66.7	60.0	0.20
TNF- α , % detectable	66.7	77.8	70.0	0.99
Cardiac troponin I, % detectable	11.1	22.2	10.0	0.82

Probability values for statistical differences in means and percentages between dosage groups were calculated with exact permutation tests. Percent detectable is the percent of animals in each group with detectable concentrations

hsCRP high-sensitivity C-reactive protein, TNF tumor necrosis factor, R1 receptor 1, IL interleukin

^aType I error rate (α) = 0.01

Table 4. Concentrations of cardiovascular biomarkers in 28 rhesus macaque monkeys after receiving methylphenidate for 5 years, by dosage group

Biomarker	Methylphenidate dosage (mg/kg, b.i.d.)			P value ^a
	Control group (0), n = 9	Low-dose group (2.5), n = 9	High-dose group (12.5), n = 10	
Glucose, mean (SD) (mg/dL)	68.4 (8.9)	62.1 (5.8)	67.6 (11.6)	0.30
Insulin, mean (SD) (uU/mL)	7.30 (6.01)	4.17 (4.15)	8.09 (13.5)	0.75
Total cholesterol, mean (SD) (mg/dL)	119 (20.1)	111 (15.2)	118 (25.1)	0.70
Triglycerides, mean (SD) (mg/dL)	37.9 (6.5)	41.1 (20.1)	46.4 (5.8)	0.53
HDL, mean (SD) (mg/dL)	66.2 (13.4)	60.3 (15.7)	60.7 (13.2)	0.62
LDL, mean (SD) (mg/dL)	45.3 (10.1)	42.8 (7.9)	48.4 (17.2)	0.61
VLDL, mean (SD) (mg/dL)	7.56 (1.33)	8.22 (4.09)	9.20 (3.71)	0.55
BMI, mean (SD) (kg/m ²)	12.68 (0.94)	12.72 ^b (0.81)	12.93 (0.56)	0.76

Probability values for statistical differences in means between dosage groups were calculated with exact permutation tests
HDL high-density lipoprotein cholesterol, LDL low-density lipoprotein cholesterol, VLDL very-low-density lipoprotein cholesterol, BMI body mass index

^aType I error rate (α) = 0.01

^bn = 8

Table 5. Concentrations of biomarkers of endothelial adhesion, fibrosis, and atherosclerosis in 28 rhesus macaque monkeys after receiving methylphenidate for 5 years, by dosage group

Serum biomarker	Methylphenidate dosage (mg/kg, b.i.d.)			P value ^a
	Control group (0), n = 9	Low-dose group (2.5), n = 9	High-dose group (12.5), n = 10	
ICAM1, mean (SD) (pg/mL)	7617 (2591)	7702 (3089)	9654 (1974)	0.56
VCAM1, mean (SD) (pg/mL)	1092 (209)	881 (326)	1294 (785)	0.24
P selectin, mean (SD) (U/mL)	87.1 (23.9)	79.9 (12.5)	78.7 (15.1)	0.55
Soluble E selectin, mean (SD) (U/mL)	56.6 (33.8)	47.2 (23.5)	46.9 (15.7)	0.66
PAI1, mean (SD) (pg/mL)	0.599 (0.237)	0.544 (0.046)	0.930 (1.192)	0.47
MCP1, mean (SD) (pg/mL)	153 (31.6)	137 (53.4)	116 (56.6)	0.28
Adiponectin, mean (SD) (μ g/mL)	14.6 (4.43)	18.43 (7.0)	19.9 (7.42)	0.21
CICP, mean (SD) (ng/mL)	165 (88.4)	163 (68.2)	172 (60.2)	0.96

Probability values for statistical differences in means between dosage groups were calculated with exact permutation tests
CAM intercellular adhesion molecule, VCAM vascular cell adhesion protein, PAI plasminogen activator inhibitor, MCP monocyte chemotactic protein, CICP collagen I carboxyterminal peptide

^aType I error rate (α) = 0.01

Table 6. Prevalence of detected lesions by microscopic analysis in 28 rhesus macaque monkeys after receiving methylphenidate for 5 years, by dosage group

Lesion	Methylphenidate dosage (mg/kg, b.i.d.)			P value ^a
	Control group (0), n = 9 Prevalence (%)	Low-dose group (2.5), n = 9 Prevalence (%)	High-dose group (12.5), n = 10 Prevalence (%)	
Myelin figure/body in the sarcoplasm	100	100	90	NE
Membrane accumulation in the sarcoplasm	100	89	80	0.75
Edema in the sarcoplasm	78	78	70	0.90
Dilution of the sarcoplasmic reticulum	89	89	80	0.81
Increased lipid droplets	33	33	40	0.94
Lipofuscin bodies	78	78	90	0.70
Hypercomplex folding in the nucleus	0	11	10	NE
Myelin figures in the extracellular space	56	33	40	0.71
Mitochondria in the extracellular space	44	44	60	0.79
Cellular infiltration of mononuclear cell, focal ^b	33	33	10	0.48
Necrosis, focal ^b	11	0	0	NE

Prevalence was 100% across all dosage groups for degenerative mitochondria, myelin figure mitochondria, increased mitochondria, and dysmorphic mitochondria. Probability values for statistical differences in percentages between dosage groups were calculated with exact permutation tests NE, not estimatable. Unless otherwise noted, all data are from transmission electron microscopy

^aType I error rate (α) = 0.01

^bLight microscopy

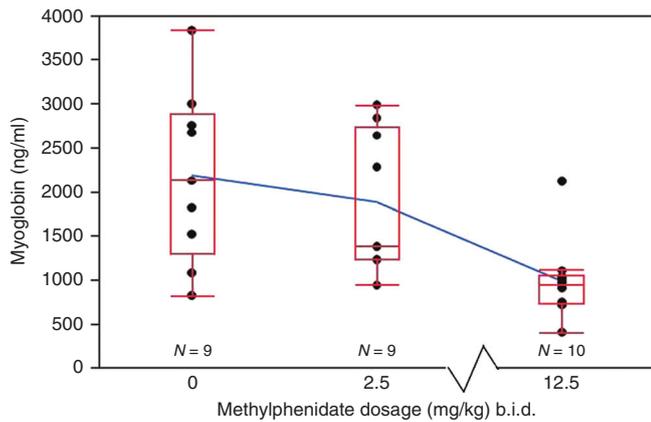


Fig. 1 Side-by-side dot plots with quantile box plot overlays of serum myoglobin concentration by methylphenidate dosage. Blue line connects group means. Overall $P = 0.0006$. P (0 versus 2.5) = 0.4779. P (0 versus 12.5) = 0.0025. P (2.5 versus 12.5) = 0.0081

one difference of statistical significance: mean serum myoglobin concentrations were significantly lower in the high-dose group. Elevated serum myoglobin levels are associated with either skeletal or cardiac muscle damage. Despite a thorough literature search, we could find no published normal values for serum myoglobin in this species or any other non-human primates. Since most established normal ranges for clinical chemistry and hematological measures in rhesus monkeys are comparable to those in humans,²⁶ we used the normal value for humans (<90 ng/mL) to interpret the serum myoglobin results from this study.²⁷ Serum myoglobin levels associated with acute myocardial infarction can be up to 500 ng/mL and return to normal within 24 h.²⁸ Serum myoglobin levels in acute skeletal muscle injury (e.g., myositis, rhabdomyolysis) range from 300 to 20,000 ng/mL.^{29,30} In the absence of demonstrated clinical or laboratory evidence of significant cardiac disease in this cohort, we concluded that the origin of the elevated serum myoglobin levels in all three groups is most likely skeletal muscle. There is one report of myoglobinuria in this species related to exertional rhabdomyolysis, which would have to be accompanied by a very elevated serum myoglobin level although the serum level was not reported.³¹ The mechanism responsible for the significantly lower, but still markedly elevated serum myoglobin level in the high-dose group, as compared to the low-dose and control groups, cannot be determined from this study. Since all three groups had markedly elevated serum myoglobin levels, based on normal human values, we do not believe that the statistically significant lower level in the high-dose group compared to the other two dosage groups is clinically important. If in the future our assumption that normal serum myoglobin levels are comparable in humans and rhesus monkeys is shown to be invalid, we will have to revisit our interpretation of the myoglobin results.

Several placebo-controlled studies have documented statistically significant but clinically unimportant increases in heart rate and/or systolic or diastolic blood pressure with both short- and long-term administration of MPH in children and adults.^{32–36} None of these studies has shown MPH to be causally related to increased risks for serious cardiac events and none of them included a cardiovascular assessment beyond blood pressure and heart rate monitoring. A large retrospective study of adults found an association between initiation of MPH therapy and a 2-fold increase in sudden death or ventricular arrhythmias.³⁷ However, the authors note that the lack of a dose–response relationship suggests the association may not be causal. The same group performed a similar study in children and adolescents with a prescription for any stimulant, including MPH, for ADHD and found no difference in serious cardiac

events, although they noted that the very low event rates in this population limited their ability to rule out relative increases in cardiac event rates.³⁸ A retrospective study by another group, using four large commercial databases, found no association between stimulant use for ADHD and serious cardiovascular events in young and middle-aged adults.³⁹ Our findings of no apparent cardiac effects associated with MPH use in this randomized, placebo-controlled study in non-human primates are consistent with most of the human studies referenced above.

Strengths and limitations of the study

The randomized trial design and carefully controlled conditions in the animals' maintenance and treatment are strengths of the study. Another strength is that a prospective study in a pre-clinical species avoids the biases and confounding associated with retrospective studies. Regarding the cardiac studies, a standard ECG cannot rule out the risk for a subsequent serious arrhythmia such as ventricular tachycardia. Although extrapolating the results from animal research to humans should always be made with some caution,⁴⁰ the non-cardiac effects of MPH have been studied in rhesus monkeys with results comparable to studies in humans.

CONCLUSIONS

Our data suggest chronic MPH exposure is not associated with clinically important evidence of cardiotoxicity. Because MPH is prescribed to more than 1.8 million children annually, it may be important to continue to monitor for adverse cardiac events even though they are likely to be rare.

ACKNOWLEDGMENTS

Andrea Parra and Katherine Kralievs provided support for the data collection and database development for this study. This study was funded by awards from the Eunice Kennedy Shriver National Institute of Child Health and Human Development, the NCTR, and the FDA. NCTR ID #: E0728711. The opinions and conclusions are those of the authors and not necessarily of the FDA.

AUTHOR CONTRIBUTIONS

Substantial contributions to conception and design: J.D.W., S.E.L., R.C., W.F.S., W.S. Acquisition of data: J.D.W., R.C., S.K.S., J.G., A.P., K.D., Y.J., D.D.D. Analysis and interpretation of data: All authors. Drafting the article: J.D.W., R.C., W.F.S., S.K.S., A.P., K.D., P.G.R., J.C., A.B., J.A.W., S.E.L. Revising it critically for important intellectual content: All authors. Final approval of the version to be published: All authors.

ADDITIONAL INFORMATION

Competing interests: The authors declare no competing interest.

Publisher's note: Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

REFERENCES

- Grace, C. et al. Trends of outpatient prescription drug utilization in US children, 2002–2010. *Pediatrics* **130**, 23–31 (2012).
- Nymark, T. B., Hovland, A., Bjørnstad, H. & Nielsen, E. W. A young man with acute dilated cardiomyopathy associated with methylphenidate. *Vasc. Health Risk Manag.* **4**, 477–479 (2008).
- Dadfarmay, S. & Dixon, J. A case of acute cardiomyopathy and pericarditis associated with methylphenidate. *Cardiovasc. Toxicol.* **9**, 49–52 (2009).
- Hole, L. D. & Schjott, J. Myocardial injury in a 41-year-old male treated with methylphenidate: a case report. *Drug Saf.* **37**, 661–676 (2014).
- Munk, K., Gormsen, L., Kim, W. Y. & Andersen, N. H. Cardiac arrest following a myocardial infarction in a child treated with methylphenidate. *Case Rep. Pediatr.* **2015**, 905097 (2015).
- Vetter, V. L. et al. Cardiovascular monitoring of children and adolescents with heart disease receiving medications for attention deficit/hyperactivity disorder

- [corrected]: a scientific statement from the American Heart Association Council on Cardiovascular Disease in the Young Congenital Cardiac Defects Committee and the Council on Cardiovascular Nursing. *Circulation* **117**, 2407–2423 (2008).
7. Perrin, J. M., Friedman, R. A. & Knilans, T. K., Black Box Working Group, Section on Cardiology and Cardiac Surgery. Cardiovascular monitoring and stimulant drugs for attention-deficit/hyperactivity disorder. *Pediatrics* **122**, 451–453 (2008).
 8. American Academy of Pediatrics/American Heart Association. American Academy of Pediatrics/American Heart Association clarification of statement on cardiovascular evaluation and monitoring of children and adolescents with heart disease receiving medications for ADHD: May 16, 2008. *J. Dev. Behav. Pediatr.* **29**, 335 (2008).
 9. Gould, M. S. et al. Sudden death and use of stimulant medications in youths. *Am. J. Psychiatry* **166**, 992–1001 (2009).
 10. Nissen, S. E. ADHD drugs and cardiovascular risk. *N. Engl. J. Med.* **354**, 1445–1448 (2006).
 11. Kuehn, B. M. Stimulant use linked to sudden death in children without heart problems. *JAMA* **302**, 613–614 (2009).
 12. Newcorn, J. H. & Donnelly, C. Cardiovascular safety of medication treatments for attention-deficit/hyperactivity disorder. *Mt. Sinai J. Med.* **76**, 198–203 (2009).
 13. Olsson, M. et al. Stimulants and cardiovascular events in youth with attention-deficit/hyperactivity disorder. *J. Am. Acad. Child Adolesc. Psychiatry* **51**, 147–156 (2012).
 14. Bange, F., Le Heuzey, M. F., Acquaviva, E., Delorme, R. & Mouren, M. C. Cardiovascular risks and management during attention deficit hyperactivity disorder treatment with methylphenidate. *Arch. Pediatr.* **21**, 108–112 (2014).
 15. Shin, J. Y., Roughhead, E. E., Park, B. J. & Pratt, N. L. Cardiovascular safety of methylphenidate among children and young people with attention-deficit/hyperactivity disorder (ADHD): nationwide self controlled case series. *BMJ* **353**, i2550 (2016).
 16. Hennissen, L. et al. Cardiovascular effects of stimulant and non-stimulant medication for children and adolescents with ADHD: a systematic review and meta-analysis of trials of methylphenidate, amphetamines and atomoxetine. *CNS Drugs* **31**, 199–215 (2017).
 17. Subcommittee on Attention-Deficit/Hyperactivity Disorder; Steering Committee on Quality Improvement and Management, Wolraich, M. et al. ADHD: clinical practice guideline for the diagnosis, evaluation, and treatment of attention-deficit/hyperactivity disorder in children and adolescents. *Pediatrics* **128**, 1007–1022 (2011).
 18. Take, G. et al. Dose-dependent immunohistochemical and ultrastructural changes after oral methylphenidate administration in rat heart tissue. *Anat. Histol. Embryol.* **37**, 303–308 (2008).
 19. Henderson, T. A. & Fischer, V. W. Effects of methylphenidate (Ritalin) on mammalian myocardial ultrastructure. *Am. J. Cardiovasc. Pathol.* **5**, 68–78 (1995).
 20. Witt, K. L. et al. No increases in biomarkers of genetic damage or pathological changes in heart and brain tissue in male rats administered methylphenidate hydrochloride (Ritalin) for 28 days. *Environ. Mol. Mutagen.* **51**, 80–88 (2010).
 21. Zhang, X. et al. MicroPet/CT assessment of FDG uptake in brain after long-term methylphenidate treatment in nonhuman primates. *Neurotoxicol. Teratol.* **56**, 68–74 (2016).
 22. Morris, S. M. et al. The genetic toxicology of methylphenidate hydrochloride in non-human primates. *Mutat. Res.* **673**, 59–65 (2009).
 23. Mattison, D. R. et al. Pubertal delay in male nonhuman primates (*Macaca mulatta*) treated with methylphenidate. *Proc. Natl Acad. Sci. USA* **108**, 16301–16306 (2011).
 24. Rodriguez, J. S. et al. The effects of chronic methylphenidate administration on operant test battery performance in juvenile rhesus monkeys. *Neurotoxicol. Teratol.* **32**, 142–151 (2010).
 25. Yang, X. et al. Development of a physiologically based model to describe the pharmacokinetics of methylphenidate in juvenile and adult humans and non-human primates. *PLoS ONE* **9**, e106101 (2014).
 26. Matsuzawa, T. & Nagai, Y. Comparative haematological and plasma chemistry values in purpose-bred squirrel, cynomolgus and rhesus monkeys. *Comp. Haematol. Int* **4**, 43–48 (1994).
 27. <https://www.mayomedicallaboratories.com/test-catalog/Clinical+and+Interpretive/35110>. Accessed 25 Feb 2018.
 28. Sallach, S. M. et al. Change in serum myoglobin to detect acute myocardial infarction in patients with normal troponin I levels. *Am. J. Cardiol.* **94**, 864–867 (2004).
 29. Keltz, E., Fahmi, Y., Khan, Y. & Mann, G. Rhabdomyolysis. The role of diagnostic and prognostic factors. *Muscles Liga. Tendons J.* **3**, 303–312 (2013).
 30. Olerud, J. E., Homer, L. D. & Carroll, H. W. Incidence of acute exertional rhabdomyolysis: serum myoglobin and enzyme levels as indicators of muscle injury. *Arch. Intern. Med.* **136**, 692–697 (1976).
 31. Reuter, J. D., Dysko, R. E. & Crisp, C. E. Review of exertional rhabdomyolysis in a rhesus monkey (*Macaca mulatta*). *J. Med. Primatol.* **27**, 303–309 (1998).
 32. Findling, R. L. et al. Short- and long-term cardiovascular effects of mixed amphetamine salts extended release in children. *J. Pediatr.* **147**, 348–354 (2005).
 33. Donner, R., Michaels, M. A. & Ambrosini, P. J. Cardiovascular effects of mixed amphetamine salts extended release in the treatment of school-aged children with attention-deficit/hyperactivity disorder. *Biol. Psychiatry* **61**, 706–712 (2007).
 34. Wilens, T. E., Biederman, J. & Lerner, M. Effects of once-daily osmotic release methylphenidate on blood pressure and heart rate in children with attention-deficit/hyperactivity disorder: results from a one-year follow-up study. *J. Clin. Psychopharmacol.* **24**, 36–41 (2004).
 35. Wilens, T. E., Spencer, T. J. & Biederman, J. Short- and long-term cardiovascular effects of mixed amphetamine salts extended-release in adolescents with ADHD. *CNS Spectr.* **10**(Suppl. 15), 22–30 (2005).
 36. Biederman, J. et al. A randomized, placebo-controlled trial of OROS methylphenidate in adults with attention-deficit disorder. *Biol. Psychiatry* **59**, 829–835 (2006).
 37. Schelleman, H. et al. Methylphenidate and risk of serious cardiovascular events in adults. *Am. J. Psychiatry* **169**, 178–185 (2012).
 38. Schelleman, H. et al. Cardiovascular events and death in children exposed and unexposed to ADHD agents. *Pediatrics* **127**, 1102–1110 (2011).
 39. Habel, L. A. et al. ADHD drugs and serious cardiovascular events in children and young adults. *JAMA* **365**, 1896–1904 (2011).
 40. Lipshultz, S. E., Cohen, H., Colan, S. D. & Herman, E. H. The relevance of information generated by in vitro experimental models to clinical doxorubicin cardiotoxicity. *Leuk. Lymphoma* **47**, 1454–1458 (2006).