Methamphetamine Use During the First or Second Half of Pregnancy Worsens Cardiac Ischemic Injury in Adult Female Offspring

Hasitha CHAVVA[1], Boyd R. RORABAUGH[1,2]

[1]Department of Pharmaceutical Sciences, Marshall University School of Pharmacy, Huntington, USA, [2]Department of Biomedical Sciences, Joan C. Edwards School of Medicine, Marshall University, Huntington, USA

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Summary

There is growing evidence that methamphetamine use during pregnancy may produce detrimental cardiovascular effects in the adult offspring. Prior work demonstrated that chronic methamphetamine exposure throughout the gestational period causes adult female offspring to become hypersensitive to myocardial ischemic injury. The goal of the present study was to determine whether this methamphetamine-induced effect occurs early or late in the gestational period. Pregnant female rats were divided into 4 experimental groups. Groups 1 and 2 received subcutaneous injections of saline (group 1) or methamphetamine (5 mg/kg) (group 2) throughout the gestational period. Group 3 received methamphetamine injections on days 1-11 and saline on days 12-22, and group 4 received saline on days 1-11 and methamphetamine on days 12-22. Hearts were isolated from adult (8 weeks) female offspring and subjected to 30 min ischemia and 2 hours reperfusion on a Langendorff isolated heart apparatus. Contractile function was measured via an intraventricular balloon, and infarct size was measured by triphenyltetrazolium chloride staining. Infarcts were significantly larger in methamphetamine exposed offspring regardless of whether they had been exposed to methamphetamine during the first half or the second half of the gestational period. Prenatal exposure to methamphetamine had no effect on preischemic contractile function or postischemic recovery of contractile function. These data indicate that methamphetamine use during either the first half or second half of pregnancy increases susceptibility to myocardial infarction in adult female offspring. These data provide further evidence that prenatal exposure to methamphetamine may increase the risk of developing cardiovascular diseases during adulthood.

Key words

Prenatal methamphetamine • Heart • Ischemia • Gestation • Pregnancy

Corresponding author

Boyd R. Rorabaugh, Marshall University School of Pharmacy, 1 John Marshall Drive, Huntington, WV 25755, USA. E-mail: rorabaughb@marshall.edu

Introduction

Substance use disorders are a major public health concern. According to the 2020 National Survey on Drug Use and Health, 59 million Americans over the age of 12 have used an illicit drug within the past year, and approximately 6 % of United States residents over the age of 12 have used methamphetamine at least once during their lifetime [1]. The Infant Development, Environment, and Lifestyle (IDEAL) study estimated that 5.2 % of pregnant women used methamphetamine during their pregnancy [2]. Approximately one-third of pregnant methamphetamine users in this study decreased their methamphetamine use during pregnancy [3]. However, most either increased (10 %) or did not change (55 %) their patterns of methamphetamine use between the first and third trimesters of pregnancy, resulting in prenatal exposure of their children to methamphetamine [3].

Most studies investigating the impact of prenatal exposure to methamphetamine have focused on neurological, behavioral, and cognitive outcomes in the offspring [4-7]. Few studies have investigated the impact

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Fax +420 241 062 164, e-mail: physres@fgu.cas.cz, www.biomed.cas.cz/physiolres

of methamphetamine use during pregnancy on the cardiovascular function of adult offspring. Work in our laboratory has demonstrated that prenatal exposure to methamphetamine has negative cardiovascular effects in the adult offspring. We previously reported that adult female rats that were prenatally exposed to methamphetamine throughout the gestational period developed myocardial hypersensitivity to ischemic injury [8]. In contrast, hearts of their adult male littermates were unaffected. More recently, we found that adult male rats that were prenatally exposed to methamphetamine demonstrate vascular dysfunction including attenuated acetylcholine-induced relaxation and potentiation of angiotensin II-induced contraction of the aorta [9]. These data suggest that prenatal exposure to methamphetamine may increase the risk of developing cardiovascular disease later in adult life.

In our previous studies, pregnant female rats were exposed to methamphetamine starting on the first day of gestation and continuing until the pups were born [8]. Thus, it was unclear whether methamphetamine-induced sensitization of the heart requires methamphetamine exposure throughout gestation or at what point during the gestational period methamphetamine induces this effect. Thus, the goal of the present study was to determine whether prenatal exposure to methamphetamine during either the first or second half of the gestational period leads to myocardial hypersensitivity to ischemia in adult female offspring.

Methods

Animals and methamphetamine treatment

Sprague-Dawley rats were purchased from Charles River Laboratories (Wilmington, MA, USA; Strain Code 001). Male and female animals were cohoused until a vaginal plug was identified. The male was then removed, and the presence of a vaginal plug was defined as gestational day 0. The rats were housed in standard cages with free access to food and water and on 12/12hr light/dark cycle (lights on at 0600). Pregnant rats were divided into four groups (Fig. 1). Group 1 received subcutaneous saline injections starting on gestational day 1 and continuing until the pups were born. Group 2 received subcutaneous methamphetamine injections (5 mg/kg) starting on gestational day 1 and continuing until the pups were born. Group 3 received subcutaneous injections of saline on gestational days 1-11 and methamphetamine starting on gestational day 12 and continuing until pups were born. Group 4 received subcutaneous injections of methamphetamine on days 1-11 and saline injections starting on day 12 and continuing until the pups were born. All injections were administered once per day at 0800. The 5 mg/kg dose of methamphetamine was used because it is reportedly within the range of methamphetamine doses typically used by people in illicit settings [10]. In addition, this dose of methamphetamine has been shown to hypersensitize the adult heart to myocardial ischemic injury [8] and is commonly used by other investigators to study the behavioral and locomotor effects of methamphetamine in rats [11-13].

Pups were weaned when they reached 28 days of age. Our prior work demonstrated that prenatal exposure to methamphetamine selectively sensitizes females to myocardial ischemic injury [8]. The hearts of male offspring are unaffected. Thus, only female offspring were used in this study. All procedures were conducted in accordance with the *Guide for the Care and Use of Laboratory Animals*. This work was approved by the Institutional Animal Care and Use Committee of Marshall University.

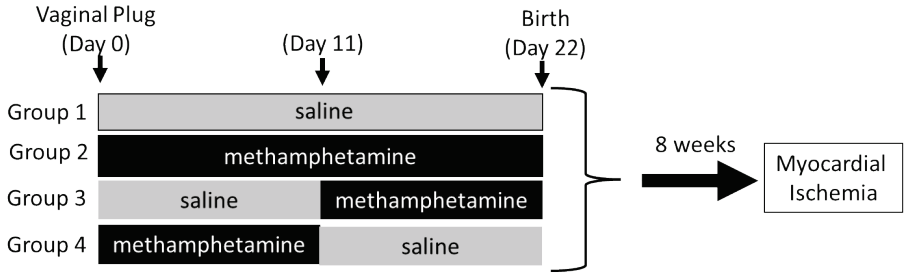

Fig. 1. Experimental groups. Pregnant rats were randomly assigned to groups 1-4. Gestational day 0 was defined as the day that a vaginal plus was identified. Group 1 and group 2 received daily subcutaneous injections of either saline or methamphetamine (5 mg/kg) starting on day 1 and continuing until the pups were born on day 22. Group 3 received injections of saline on days 1-11 and methamphetamine on days 12-22. Group 4 received injections of methamphetamine on days 1-11 and saline on days 12-22. Hearts of female offspring were subjected to 30 min ischemia and 2 hours reperfusion on a Langendorff isolated heart apparatus when the animals reached 8 weeks of age.

Langendorff isolated heart experiments

Female offspring were anesthetized with sodium pentobarbital (150 mg/kg) when they reached 8 weeks of age. The heart was quickly isolated and mounted on a Langendorff isolated heart apparatus as previously described [8]. Krebs solution (in mM: 118 NaCl, 4.7 KCl, 1.2 $MgSO_4$, 25 $NaHCO_3$, 1.2 KH_2PO_4, 0.5 Na_2EDTA, 11 glucose, and 2.5 $CaCl_2$, pH 7.4) was perfused at a constant pressure of 80 mm Hg through an aortic cannula. The contractile function of the left ventricle was measured using an intraventricular balloon connected to a pressure transducer and inflated to an end diastolic pressure of 4-8 mmHg. Cardiac contractile function was recorded using a Powerlab 4SP data acquisition system (AD Instruments, Colorado Springs, CO). The heart was maintained at a temperature of 37 °C throughout the experiment, and the temperature of the heart was continuously monitored using a thermocouple placed on the surface of the heart. Hearts were equilibrated for 25 min prior to the onset of 30 min of ischemia followed by 2 hours of reperfusion. Hearts were excluded after the 25 min equilibration period if developed pressure was less than 70 mmHg, coronary flow rate was greater than 15 ml/min (indicative of a leak or damaged vasculature), or if there were persistent arrhythmias.

Measurement of infarct size

Infarct size was measured as previously described [8]. Hearts were perfused with 1% triphenyl tetrazolium chloride and then submerged in 1% triphenyl tetrazolium chloride for 12 min at 37 ºC. Hearts were subsequently frozen, sliced into 1 mm sections, soaked in 10% neutral buffered formalin, and then photographed with a Nikon SMZ 800 microscope equipped with a Nikon DS-Fi1 digital camera. Image J software was used to measure the area of infarcted tissue and the total surface area of each slice. Infarct size was expressed as a percentage of the entire ventricular myocardium.

Statistical analysis

Infarct size was measured by one-way ANOVA and Tukey's posthoc analysis. Parameters of cardiac contractile function (developed pressure, +dP/dT, -dP/dT, and heart rate) and coronary flow rate were analyzed by two way ANOVA (factors = time and drug treatment) with time as a repeated measure. P values < 0.05 were considered statistically significant. A total of 10-12 animals were included in each experimental group.

Results

Prenatal methamphetamine had no impact on litter size or body weight of adult offspring

Methamphetamine had no impact on litter size (Group 1: 13 ± 1 pups; Group 2: 12 ± 1 pups; Group 3: 13 ± 1 pups; Group 4: 11 ± 1 pups). Body weight at 8 weeks of age was also unaffected by prenatal exposure to methamphetamine (Group 1: 234 ± 7 g; Group 2: 224 ± 7 g; Group 3: 235 ± 9 g; Group 4: 251 ± 4 g).

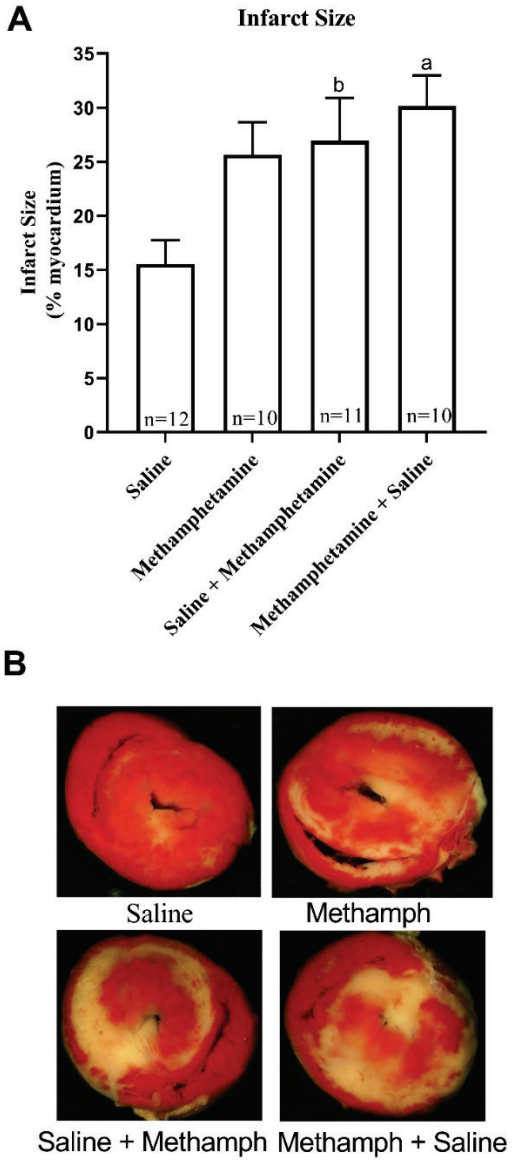

Fig. 2. Methamphetamine exposure during either the first half or second half of the gestational period increases infarct size following an ischemic insult in adult female offspring. One-way ANOVA indicated a significant effect of methamphetamine on infarct size [$F = 4.5$ (3,39), $p < 0.01$] (**A**). Representative images of triphenyltetrazolium chloride-stained hearts are shown in panel **B**. [a] indicates $p < 0.01$ compared to hearts from animals exposed to prenatal saline. [b] indicates $p < 0.05$ compared to hearts from animals exposed to prenatal saline.

Methamphetamine exposure during either the first half or second half of gestation increases infarct size following an ischemic insult

One-way ANOVA indicated a significant effect [F = 5 (3, 39), p < 0.05] of methamphetamine on infarct size in adult female hearts (Fig. 2.). Tukey's posthoc analysis indicated that infarcts were significantly larger in hearts isolated from rats that had been exposed to methamphetamine during either the first half (group 4) or the second half (group 3) of the gestational period compared to those which were treated with saline throughout gestation (group 1). Exposure to methamphetamine throughout the gestational period (group 2) caused a 31 % increase in infarct size compared to exposure to saline-treated control animals. However, this effect did not reach statistical significance. In addition, infarct sizes in hearts from animals exposed to methamphetamine throughout the gestational period (group 2) were not significantly different from those exposed to methamphetamine during either the first (group 4) or second (group 3) half of gestation.

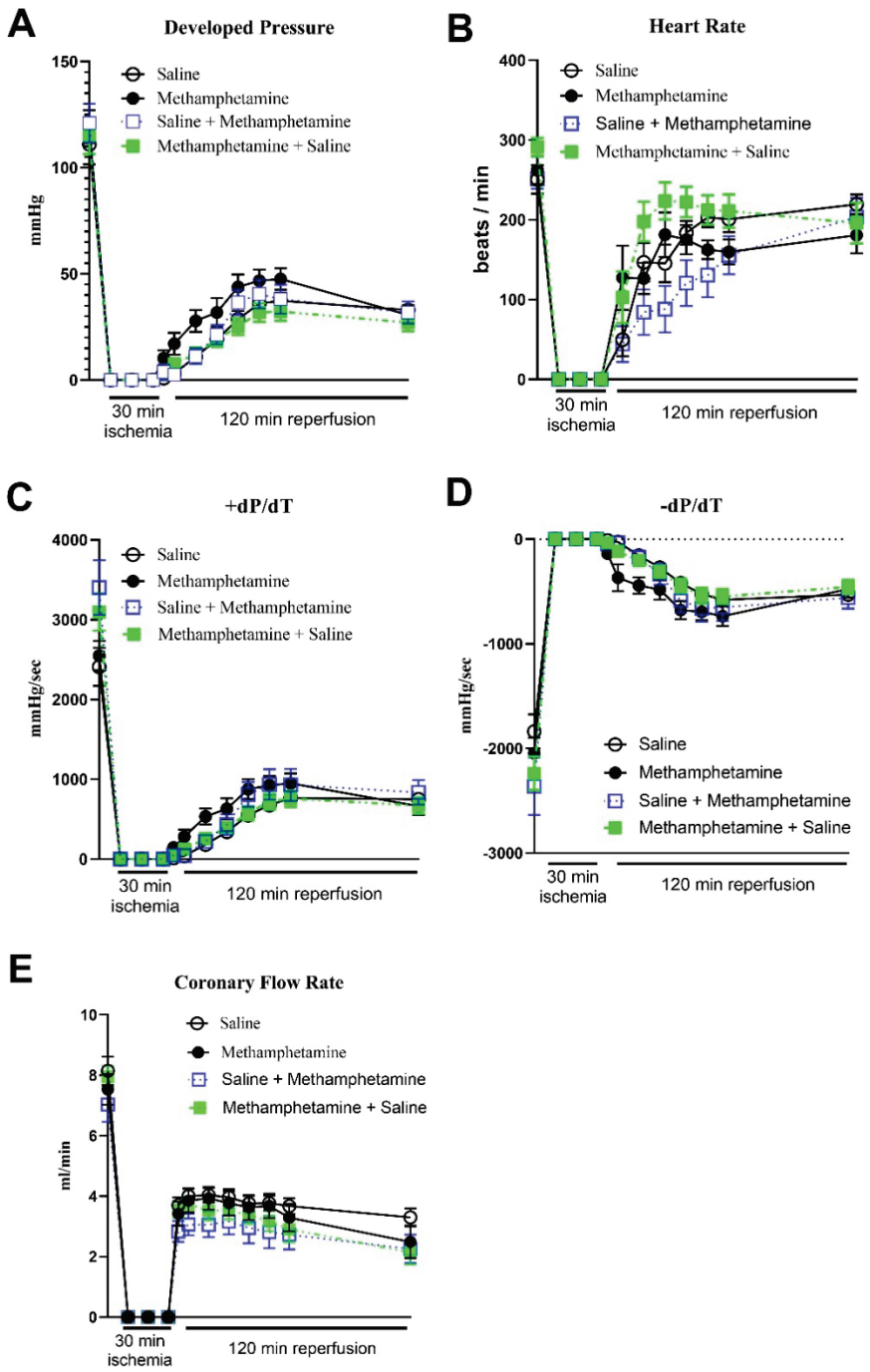

Fig. 3. Prenatal exposure to methamphetamine had no effect on post-ischemic recovery of contractile function. Postischemic developed pressure [F = 232 (3, 89), p < 0.0001] (**A**), heart rate [F = 36 (5, 131), p < 0.0001] (**B**), +dP/dT [F = 243 (2, 62), p < 0.001] (**C**), -dP/dT [F = 238 (2, 69), p < 0.0001] (**D**), and coronary flow rate [F = 239 (3, 73), p < 0.0001] (**E**) were significantly decreased relative to their respective preischemic values. There was also a significant interaction between time and methamphetamine treatment for heart rate [F = 2 (33, 312), p = 0.01] (**B**), +dP/dT [F = 3 (33, 304), p < 0.0001] (**C**), and -dP/dT [F = 2 (33, 312), p < 0.05] (**D**), but there was no significant effect of prenatal methamphetamine on these parameters of cardiac function. Data represent the mean ± S.E.M of 10-12 rats for each group.

Two-way ANOVA indicated significant effects of time (before ischemia vs postischemic recovery) on developed pressure [$F = 232$ (3, 89), $p < 0.0001$] (Fig. 3A), heart rate [$F = 36$ (5, 131), $p < 0.0001$] (Fig. 3B) +dP/dT [$F = 243$ (2, 62), $p < 0.001$] (Fig. 3C), -dP/dT [$F = 238$ (2, 69), $p < 0.0001$] (Fig. 3D), and coronary flow rate [$F = 239$ (3, 73), $p < 0.0001$] (Fig. 3E). There was also a significant interaction between time and methamphetamine treatment for heart rate [$F = 2$ (33, 312), $p = 0.01$] (Fig. 3B), +dP/dT [$F = 3$ (33, 304), $p < 0.0001$] (Fig. 3C), and -dP/dT [$F = 2$ (33, 312), $p < 0.05$] (Fig. 3D) reflecting the fact that these parameters of cardiac function were significantly decreased following ischemia. However, prenatal exposure to methamphetamine did not cause a further worsening of postischemic contractile recovery.

Discussion

Previous work demonstrated that methamphetamine exposure throughout the gestational period leads to myocardial hypersensitivity to ischemic injury in adult female offspring [8]. The current study extends these findings by demonstrating that fetal exposure to methamphetamine during either the first half or the second half of the gestational period causes the adult female heart to become hypersensitive to ischemia. Della Grotta et al. [3] reported that 35 % of women that used methamphetamine prior to becoming pregnant decreased their methamphetamine consumption over the course of their pregnancy. Our data suggest that terminating methamphetamine use midway through a pregnancy may be insufficient to avoid this detrimental cardiac effect in the female offspring.

Methamphetamine exposure during either the first (Group 4) or second (Group 3) half of gestation resulted in a statistically significant increase in myocardial infarct size (Fig. 2). Prenatal methamphetamine exposure throughout the entire gestational period (Group 2) resulted in a 31 % increase in infarct size, but this did not reach statistical significance when assessed by Tukey's posthoc test. In light of our prior work [8] and Groups 3 and 4 in the present study, caution is warranted in concluding that methamphetamine exposure throughout the entire gestational period (Group 2) produces an effect on the heart that is physiologically distinct from the effect that results from methamphetamine exposure limited to either the first (Group 4) or second half (Group 3) of gestation. More importantly, our data provide evidence that daily methamphetamine use at either the beginning or end of pregnancy sensitizes the hearts of female offspring to ischemia.

Methamphetamine is associated with a significant risk of addiction and physiological dependence. Withdrawal symptoms occur when a physiologically dependent individual experiences subsequent abstinence from methamphetamine. Withdrawal symptoms in humans include fatigue, depression, dysphoria, and methamphetamine cravings. Rodent studies of physiological dependence typically employ self-administration models of escalating doses of methamphetamine, and behavioral assays (lever pressing, nose poking, preference for novel objects, locomotor activity, etc.) are used to assess dependence and withdrawal-related behaviors induced by subsequent abstinence from methamphetamine [14,15]. Behavioral assessments of physiological dependence or withdrawal were not used in pregnant dams or neonatal pups in the present study. Thus, it is unclear whether the dams or pups developed physiological dependence or experienced withdrawal symptoms when they subsequently became abstinent from methamphetamine at birth (Groups 2 and 3) or after gestational day 11 (Group 4). We are unaware of any evidence that abstinence-induced withdrawal from methamphetamine or other CNS stimulants results in physiological effects that persist in the hearts of adult offspring. However, we cannot exclude the possibility that abstinence-induced withdrawal (rather than the direct actions of methamphetamine) may have contributed to the effect observed in the hearts of adult offspring.

Although infarct size was significantly increased in adult offspring that had been prenatally exposed to methamphetamine, methamphetamine exposure had no effect on basal parameters of contractile function or on postischemic recovery of contractile function (Fig. 3.). This is consistent with prior work from our own lab as well as the work of others that have reported that postischemic recovery of contractile function does not always correlate with changes in infarct size [8,16-18]. Some investigators have attributed this disparity to myocardial stunning in which the tissue is not dead but does not contract appropriately [16]. In addition, coronary flow rate is significantly reduced during the reperfusion period. This reduction in the delivery of oxygen and nutrients can confound measurements of the contractile capacity of the heart. For these reasons, infarct size is regarded as more reliable than postischemic recovery of

contractile function as a measure of cardiac injury [16,19].

Human methamphetamine use patterns vary widely with some individuals using methamphetamine daily and others having less frequent binges. In addition, human methamphetamine consumption is commonly accompanied by the use of alcohol, cocaine, marijuana, opioids, or other drugs. In contrast, the pregnant rats in the present study received daily methamphetamine injections in regular 24 hour intervals and were not exposed to other drugs. Furthermore, the half-life of methamphetamine is significantly longer in humans (10-15 hours) [20] than in rats (70 min) [21] making it difficult to reproduce the human pharmacokinetic profile of methamphetamine in a rat model. We do not know whether binge patterns of methamphetamine use (rather than daily dosing at regular intervals) during pregnancy produces the same result in female offspring. It is also unknown whether this methamphetamine-induced effect in the prenatal rat heart also occurs in humans that are exposed to methamphetamine during the gestational period.

Young adult (8 weeks old) rats were used in this study to be consistent with our previous work [8] and with the work of other investigators that have conducted similar studies with prenatal exposure to other CNS stimulants such as cocaine [22] and nicotine [23]. However, ischemic heart disease primarily effects the geriatric population. Our data demonstrate that the effects of prenatal exposure to methamphetamine persist following at least two months of postnatal abstinence from methamphetamine. However, it is unknown whether this effect persists into the geriatric phase of life when ischemic heart disease is most common. Further work is needed to determine whether myocardial hypersensitivity to ischemia persists throughout the normal life span of the animals.

The detrimental cardiovascular consequences of methamphetamine use during pregnancy are not limited to the heart. We recently reported vascular dysfunction in adult rats following prenatal exposure to methamphetamine [9]. This was characterized by attenuated vasorelaxation in response to acetylcholine, potentiation of angiotensin II-induced vasoconstriction, and dysfunction of perivascular adipose tissue. Others have reported similar findings in adult rodents following prenatal exposure to cocaine [24], nicotine [25,26], and caffeine [27], suggesting that CNS stimulants that act through diverse mechanisms can lead to adverse cardiovascular outcomes in adult offspring [28].

In summary, these data demonstrate that prenatal exposure to methamphetamine during either the first or second half of the gestational period causes female offspring to be more susceptible to cardiac injury during an ischemic insult. This work provides additional evidence that methamphetamine use during pregnancy may increase the risk of developing cardiovascular disorders in adult life.

Conflict of Interest

There is no conflict of interest.

Acknowledgements

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