Modest maternal caffeine exposure affects developing embryonic cardiovascular function and growth

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Maternal caffeine exposure affects developing embryonic cardiovascular function and growth. Am J Physiol Heart Circ Physiol 294: H2248–H2256, 2008. First published March 21, 2008; doi:10.1152/ajpheart.91469.2007.—Caffeine consumption during pregnancy is reported to increase the risk of spontaneous abortion and birth defects. In the present study, we tested the hypothesis that modest maternal caffeine exposure affects developing embryonic cardiovascular function and growth without altering maternal hemodynamics. Caffeine (10 mg·kg⁻¹·day⁻¹ subcutaneous) was administered daily to pregnant CD-1 mice from embryonic days (EDs) 9.5 to 18.5 of a 21-day gestation. We assessed maternal and embryonic cardiovascular function at baseline and at peak maternal serum caffeine concentration using high-resolution echocardiography on EDs 9.5, 11.5, 13.5, and 18.5. Maternal caffeine exposure did not influence maternal body weight gain, maternal CV function, or embryo resorption. However, crown–rump length and body weight were reduced in maternal caffeine treated embryos by ED 18.5 (P < 0.05). At peak maternal serum caffeine concentration, embryonic carotid artery, dorsal aorta, and umbilical artery flow initially decreased from baseline at ED 11.5 (P < 0.05). By ED 13.5, embryonic aortic and umbilical artery flows were insensitive to the peak maternal caffeine concentration; however, the carotid artery flow remained affected. By ED 18.5, baseline embryonic carotid artery flow increased and descending aortic flow decreased versus non-caffeine-exposed embryos. Maternal treatment with the adenosine A₂A receptor inhibitor reproduced the embryonic hemodynamic effects of maternal caffeine exposure. Adenosine A₂A receptor gene expression levels of ED 11.5 embryo and ED 18.5 uterus were decreased. Results suggest that modest maternal caffeine exposure has adverse effects on developing embryonic CV function and growth, possibly mediated via adenosine A₂A receptor blockade.

CAFFEINE IS A NATURALLY OCCURRING compound contained in many beverages, foods, and medications and is frequently consumed as a central nervous system stimulant. Human caffeine intake has increased in all age groups for the last two decades and 68–74% of pregnant women consume caffeine at an average intake of 125–193 mg/day (14). Caffeine metabolism becomes slower in pregnancy, and ingested caffeine easily crosses the placenta (1, 11, 18). Although it has been suggested that the risk of fetal toxicity from caffeine in humans is low, several studies have shown that moderate to heavy caffeine consumption increases the risk of spontaneous abortion or low birth weight (13, 17, 21, 22, 26, 32, 36). Moreover, recent studies identified a dose-dependent increase in the risk of spontaneous abortion in women who ingested at least 100 mg of caffeine daily (9, 45). The pharmacokinetics of caffeine and caffeine metabolites determine their in vivo effect. Caffeine reversibly and competitively binds adenosine receptors within the range of daily human caffeine consumption, whereas higher toxic caffeine doses inhibit cyclic nucleotide phosphodiesterases and induce intracellular calcium release via ryanodine receptor (15). The majority of previous animal studies on caffeine pharmacokinetics have used much higher caffeine doses than typically consumed by humans (30–150 mg·kg⁻¹·day⁻¹) (8, 15, 20, 29–31). Therefore, it remains unclear how modest maternal caffeine intake influences in utero embryonic cardiovascular (CV) function and growth.

Recent technical advances in high-resolution ultrasound imaging now allow the longitudinal and noninvasive investigation of early murine embryonic CV function (33, 38, 51). In the present study, we investigated whether modest, recurrent maternal caffeine exposure affects developing embryonic CV function and growth in a murine animal model.

MATERIALS AND METHODS

Animals. Ten- to twelve-week-old virgin female CD-1 mice were mated with CD-1 males (2:1) overnight and examined for copulatory plugs the next morning. We defined embryonic day (ED) 0.5 as noon of the day a plug was noted. All animal use and experimental protocols were approved by the Animal Research and Care Committee of Children’s Hospital of Pittsburgh.

Maternal caffeine and adenosine receptor antagonist treatment. Saline (sham group: 24 mothers) or caffeine (10 mg·kg⁻¹·day⁻¹ per dose; caffeine group: 25 mothers) was administered daily to pregnant mothers from ED 9.5 to ED 18.5 via subcutaneous injection in the maternal nuchal fold. To determine whether the hemodynamic effects of caffeine were due to adenosine receptor inhibition, we investigated the ED 11.5 maternal and embryonic hemodynamic responses to adenosine receptor blockade using the adenosine A₁ receptor agonist 8-cyclopentyl-1,3-dimethylxanthine (CPT) or the adenosine A₂A receptor antagonist 3-(3-hydroxypropyl)-8-(m-methoxyxystyryl)-7-methyl-1-propargylxanthine phosphate disodium salt (MSX-3). We administered CPT (CPT group: 4.8 mg/kg per dose), MSX-3 (MSX-3 group: 3.0 mg/kg per dose), or simultaneous MSX-3 and caffeine (MSX-3 + caffeine group) from ED 9.5 to ED 11.5, similar to the daily dosing regimen of caffeine. The doses of the adenosine receptor antagonists were chosen based on a previously published study (37). All drugs were dissolved in sterile saline (with a few drops of 0.1 N HCl) and administered by subcutaneous injection.

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NaOH for CPT and MSX-3; final pH was adjusted to 7.4), and the injection volume was adjusted as 0.01 ml/g of maternal body weight.

Maternal serum caffeine concentration. It was technically difficult to obtain blood samples at different time points from the same animal. Therefore, we prepared a separate group of animals for each time point. Pregnant mice were treated daily with either caffeine or saline from ED 9.5 to ED 13.5. Animals were euthanized at ED 13.5 before (n = 5), 30 min after (n = 4), and 60 min after (n = 5) maternal caffeine administration. Euthanasia was performed using 5% isoflurane anesthesia followed by intracardiac injection via the left ventricular apex of a cardiac arrest solution containing a high potassium concentration (60 mM KCl). Blood samples were collected from the left ventricle before cardiac arrest solution injection. Sham animals (n = 5) were euthanized 30 min after saline injection. Serum caffeine concentrations were measured using a high-performance liquid chromatography (HPLC) system (model P-900 series; Amersham Biosciences, Piscataway, NJ) with a reversed-phase HPLC column (Sephasil Protein C18-ST4.6/250; Amersham Biosciences) (6).

Maternal and embryonic echocardiography and embryo growth assessment. Standard transthoracic (mother) and maternal transabdominal (embryo) echocardiography were performed before (baseline) and 30 min after (peak maternal serum concentration) drug administration. We used a high-frequency ultrasound imaging system with a 40-MHz mechanical sector transducer (Vevo 660; VisualSonics, Toronto, Canada) from ED 9.5 to ED 13.5 and a 13-MHz phased array transducer (Acuson Sequoia C256; Siemens Medical Solution, Malvern, PA) at ED 18.5. Mice were anesthetized with isoflurane gas (2% isoflurane induction for 1 min followed by 1.5–1.7% for maintenance) and were restrained in the supine position (41). Mouse body temperature was maintained at ~37°C with a temperature controller (THM 1000; VisualSonics) and a radiant heat lamp. Echocardiography was completed within 20 min per animal. We measured maternal heart rate (HR), left ventricular (LV) cavity dimensions, shortening fraction (SF), and estimated maternal cardiac output (CO; ml/min) using a cubic method (5). Embryonic HR and local dorsal (descending) aorta, internal carotid artery, and umbilical artery flow velocities were measured using pulsed-Doppler velocimetry, and the velocity-time integral (VTI; cm) was calculated (Fig. 1, A–D). We estimated regional embryonic arterial blood flow as VTI × HR (cm/min). We calculated embryonic ventricular fractional shortening at ED 11.5 from ventricular end-diastolic (EDD) and end-systolic (ESD) dimensions measured from M-mode images (Fig. 1E). To evaluate embryo resorption rate and embryonic growth, we euthanized mothers randomly selected from control and caffeine groups on ED 13.5 or 18.5 (n = 7 individual mothers in sham and n = 7 individual mothers in caffeine group at each embryonic day). We measured embryonic crown-rump length, anterior-posterior head length, and forelimb length. We also measured embryonic wet body weight and placenta wet weight at ED 18.5.

Real-time RT-PCR analysis for adenosine A2A receptor. We determined adenosine A2A receptor gene expression in pooled tissues from embryos, placenta, and uterine muscle at EDs 11.5 and 18.5. Total RNA was prepared using Trizol solution (Invitrogen, Carlsbad, CA) and treated with TURBO DNA-free kit (Ambion, Austin, TX) and treated with TURBO DNA-free kit (Ambion, Austin, TX). Aden-
osine A2A receptor primers were obtained from Qiagen Quanti-Tect primer assay with the target fragment sizes ~100 base pairs. For real-time analysis, one-step RT-PCR SYBR green kit (Qiagen, Valencia, CA) was used. RT-PCR amplification was performed using a MX3000P system (Stratagene, La Jolla, CA). The quantitative RT-PCR (qRT-PCR) was performed in a total volume of 25 μl containing 100 ng of total RNA. The qRT-PCR protocol used 30 min at 50°C for RT, 15 min 95°C for denaturation, 40 cycles of amplification and temperature annealing at 58°C, and extension at 72°C. A DNA dissociation curve was obtained for all samples to confirm specificity of the amplification product. To obtain relative copy number data, PCR fragments were subcloned into pCR4-TOPO vector (Invitrogen), confirmed by sequencing (University of Pittsburgh Gene Sequence Core Facilities), and then linearized for generation of standard curves. Linearized target sequence dilutions were included in each experiment to allow calculation of relative copy number. The correlation coefficient and amplification efficiencies were calculated using MX3000P software. The correlation coefficient was always >0.98, and PCR efficiency was between 93 and 100% in all experiments. The internal control β-actin was used for normalization of qRT-PCR results.

Statistical analysis. Data are means ± SD. Maternal hemodynamic data and body weight change were analyzed using two-way repeated-measures analysis of variance (ANOVA) with Tukey’s test. It was technically difficult to track blood flows longitudinally for each embryo; therefore, we pooled all measured embryonic flow data and performed two-way ANOVA followed by Tukey’s test to determine statistical differences between the experimental groups. Maternal and embryonic CV effects at the peak maternal serum caffeine concentration and adenosine antagonists were analyzed by expressing data as the percentage of change above the average daily baseline value. For real-time RT-PCR analysis, we performed two-way ANOVA with Tukey’s test. Statistical significance was defined as P < 0.05. All calculations were performed using SigmaStat (Systat Software, Point Richmond, CA).

RESULTS

Maternal serum caffeine concentration and maternal body weight gain. Maternal serum caffeine concentration, measured at baseline (trough state), 30 min, and 60 min after the caffeine administration on ED 13.5, were 0, 1.1 ± 0.27, and 1.1 ± 0.25 μg/ml, respectively. Caffeine was not detected in sham-treated mice. Caffeine did not influence maternal body weight gain (Table 1).

Embryo resorption rate and growth. Maternal caffeine exposure did not influence embryonic resorption rate determined at either ED 13.5 or ED 18.5. Although ED 13.5 crown-rump length, anterior-posterior head length, and forelimb length were similar in sham and caffeine groups, ED 18.5 crown-rump length, forelimb length, and wet body weight of caffeine-treated embryos were smaller than those of sham embryos (Table 2). Of note, placental wet weight at ED 18.5 in caffeine-treated embryos was larger than that in sham embryos (Table 2).

Effect of caffeine on baseline maternal and embryonic hemodynamics. Maternal HR was similar in caffeine and sham groups throughout gestation (Fig. 2A). By ED 18.5, maternal LV EDD increased and fractional shortening remained constant, consistent with increased maternal cardiac output in both sham and caffeine-treated groups (Fig. 2, B–D). Baseline embryonic HR, carotid artery flow, dorsal aorta flow, and umbilical artery flow increased with gestation (Fig. 3; n = 95 embryos from 24 individual mothers in sham group and n = 95 embryos from 25 individual mothers in caffeine group). Compared with sham group, maternal caffeine treatment increased baseline embryonic carotid artery flow at ED 18.5 whereas baseline dorsal aorta flow decreased, suggesting recurrent maternal caffeine exposure altered blood flow distribution within the developing embryo (Fig. 3, B and C). The umbilical artery flow at ED 18.5 was similar in both sham and caffeine groups (Fig. 3D).

Maternal and embryonic hemodynamic responses to peak maternal serum concentration. In a separate experimental group, we investigated the acute response of maternal and embryonic hemodynamics to maternal caffeine exposure (10 mg/kg) in ED 11.5 embryos (6 individual mothers with 21 embryos at baseline and 3 individual mothers with 12 embryos at 90 and 180 min after caffeine administration, respectively). Maternal caffeine administration decreased embryonic dorsal aorta flow, carotid artery flow, and LV SF, with the largest decrease occurring 30 min after caffeine administration (Fig. 4). All hemodynamic data returned to baseline by 180 min. Figure 5 shows the percent changes in maternal and embryonic hemodynamic parameters 30 min after daily caffeine administration (peak maternal serum caffeine concentration). Caffeine dosing did not alter maternal CV function or embryonic HR at peak maternal serum caffeine concentration (Fig. 5, A–C). Embryonic blood flow did not change at ED 9.5 at peak maternal serum caffeine concentration; however, by ED 11.5, embryonic carotid artery flow, dorsal aorta flow, and umbilical artery blood flow decreased significantly at peak maternal serum caffeine concentration (Fig. 5, D–F). By ED 13.5, embryonic aortic flow and umbilical artery flow were insensitive to the peak maternal serum caffeine concentration; however, caffeine exposure continued to reduce carotid artery flow at EDs 13.5 and 18.5 (Fig. 5F).

Maternal and embryonic hemodynamic effects of adenosine receptor blockade at ED 11.5. Figure 6 compares the maternal and embryonic hemodynamic responses 30 min after administration of either caffeine, the adenosine A1 receptor antagonist CPT (8 individual mothers with 36 embryos), the adenosine A2A receptor antagonist MSX-3 (8 individual mothers with 35 embryos), or simultaneous MSX-3 and caffeine (6 individual mothers with 56 embryos) at ED 11.5. Maternal hemodynamics did not change following drug administration in any groups (Fig. 6, top). Maternal CPT administration did not reduce embryonic arterial blood flows or SF. In contrast, maternal caffeine and maternal MSX-3 administration resulted in comparable decreased embryonic SF, dorsal aorta blood flow, internal carotid arterial blood flow, and umbilical arterial flow (Fig. 6, bottom). To confirm that caffeine administration mediated the embryonic hemodynamic response via adenosine A2A receptor inhibition, we measured embryonic hemody-

<table>
<thead>
<tr>
<th>Table 1. Maternal body weight</th>
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<tr>
<td>Embryonic Day</td>
</tr>
<tr>
<td>----------------</td>
</tr>
<tr>
<td>9.5</td>
</tr>
<tr>
<td>11.5</td>
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<tr>
<td>13.5</td>
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<td>18.5</td>
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Data are means ± SD. Experimental numbers were n = 24 in sham group and n = 25 in caffeine group, respectively. Maternal body weight increased during development in both sham and caffeine groups (P < 0.05, ANOVA). There was no statistically significant difference between sham and caffeine treatment groups in each embryonic day (ANOVA).
Table 2. Embryo morphology

<table>
<thead>
<tr>
<th>Embryonic Day</th>
<th>Sham</th>
<th>Caffeine</th>
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<tbody>
<tr>
<td></td>
<td>ED 13.5</td>
<td>ED 18.5</td>
</tr>
<tr>
<td>Resorption rate, %</td>
<td>5 (4/80)</td>
<td>2 (291)</td>
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<tr>
<td>Crown-rump length, mm</td>
<td>9.63±0.78 (76)</td>
<td>9.56±0.60 (89)</td>
</tr>
<tr>
<td>AP head length, mm</td>
<td>5.52±0.40 (76)</td>
<td>5.66±0.40 (89)</td>
</tr>
<tr>
<td>Forelimb length, mm</td>
<td>3.04±0.44 (76)</td>
<td>3.12±0.38 (89)</td>
</tr>
<tr>
<td>Body weight, g</td>
<td>0.19 (63)</td>
<td>0.19 (87)</td>
</tr>
<tr>
<td>Placenta weight, mg</td>
<td>95±19 (63)</td>
<td>100±18 (87)</td>
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Data are means ± SD at embryonic days (ED) 13.5 and 18.5. Resorption rate is expressed as a percentage, with numbers in parentheses indicating the number of resorbed embryos to the total number of observed embryos. Remaining values in parentheses are the number of embryos for each parameter. AP: anteroposterior. Resorption rate was not changed in both EDs. Crown-rump length, AP head length, and forelimb length increased during development in both sham and caffeine groups (*P < 0.05, ANOVA). ED 18.5 crown-rump length, forelimb length, and wet body weight of caffeine-treated embryos were smaller than those of sham embryos (‡P < 0.05, t-test). ED 18.5 placenta weight of caffeine group was larger than that of sham group (†P < 0.05, ANOVA post hoc test).

DISCUSSION

The main findings of the present study are as follows: 1) modest daily maternal caffeine exposure altered regional developing embryonic arterial blood flow and induced intrauterine growth retardation without impacting maternal CV function or weight gain; 2) caffeine at peak maternal serum concentration transiently reduced embryonic carotid arterial flow to a greater extent than dorsal (and descending) aortic or umbilical arterial flow; 3) maternal adenosine A2A receptor blockade reproduced the embryonic hemodynamic effects of maternal caffeine exposure; and 4) adenosine A2A receptor gene expression in both the uterus and developing embryo were downregulated by maternal caffeine exposure.

Despite the general concern about the effects of maternal caffeine and active caffeine metabolites on fetal well being, there is very little information on the effects of maternal caffeine on the in utero mammalian embryonic CV function during the critical periods of cardiac morphogenesis and adaptive growth. Previous reports concerning caffeine-induced hemodynamic changes in the in vivo mammalian fetus have been limited to later gestation (second and third trimesters in human) (3, 10, 25, 29, 35). In the present study, using a murine model, we found that modest maternal caffeine exposure had negative embryonic hemodynamic effects during the period of primary cardiac morphogenesis (first trimester) and that fetal growth restriction occurred during the post-cardiac morphogenesis period (second trimester). The developmental stages of mouse embryos in the present study correspond to 3- to 6-wk-old to 24- to 28-wk-old human fetuses (39). Although the hemodynamic effects of acute caffeine exposure of embryos during the primary cardiac morphogenesis period have been studied in ovo chick embryos (20) and in vitro rat embryos (30), these studies did not determine the effects of caffeine on the in utero mammalian embryo exposed to the effects of caffeine on the maternal and uteroplacental circulations.

Caffeine metabolism varies between species and the half-life of caffeine ranges from 0.7 to 1.2 h in rodents to ~3 h in humans (4, 16). Peak serum caffeine concentration is reached between 15 and 120 min after oral ingestion in humans (15). Because caffeine rapidly crosses the placenta, fetal caffeine levels appear to rapidly equilibrate with maternal levels (16, 18, 24, 46). Ingestion of a single cup of coffee (1–2 mg/kg of caffeine) results in peak maternal blood concentrations of 1–2 µg/ml within 30 min (8). Fredholm et al. (15) found that ingestion of 10 mg/kg of caffeine in rats corresponded to 3.5 mg/kg of caffeine in humans, an amount equivalent to 2 cups (16 ounces) of regular coffee. In the present study, a single dose of caffeine (10 mg/kg) produced a peak murine maternal serum concentration of 1.1 µg/ml at 30 and 60 min after caffeine administration, a level corresponding to the level measured in humans after consumption of one to two cups of regular coffee. In a preliminary study, we found that more than 30 mg/kg caffeine administration significantly increased maternal HR within 20 min (>20% from baseline, P < 0.05 ANOVA), whereas 10 mg/kg caffeine administration had no maternal HR effect (no maternal CV toxicity of caffeine) up to 80 min after caffeine administration. Therefore, we completed the current study using a daily maternal caffeine dose consistent with modest daily human consumption.

Although it is clear that a modest maternal caffeine dose significantly reduced embryonic arterial blood flow, it is important to note that this may occur via several interdependent mechanisms. The first mechanism to consider is embryonic arterial vasoconstriction. The embryonic arterial vasculature rapidly regulates vascular resistance to maintain arterial pressure at the "expense" of blood flow (50). Direct caffeine...
blockade of adenosine A2A receptor may change embryonic vascular tone via vasoconstriction with secondary decreases in blood flow. Second, total embryonic blood volume is sensitive to placental water transfer (40). Tsai et al. (43) demonstrated that adenosine acts on the human uterine artery via the A2 receptor and causes uterine artery relaxation. Moreover, caffeine has been shown to significantly decrease uterine arterial flow in pregnant rats (24) and intervillous placenta blood flow in pregnant humans (25). Thus caffeine may decrease uteroplacental blood flow with a corresponding reduction in the blood volume of the fetoplacental circulation and subsequent decrease in embryonic CV function (44). Third, placental maturation and a progressive fall in placental vascular resis-

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Fig. 2. Caffeine effects on maternal cardiovascular (CV) function. Maternal heart rate (HR) did not change with repeated caffeine dosing (A). The maternal end-diastolic left ventricular cavity dimension (LV-IDD; B) and the cardiac output (CO; D) increased in parallel with embryonic day (*P < 0.05, ANOVA) without changing LV shortening fraction (LV-SF; C). No significant difference was found between the sham group and the caffeine group. Values are means ± SD; bpm, beats/min.

Fig. 3. Caffeine effects on baseline developing embryonic CV function. The embryonic HR (A) and all arterial flows (B–D) increased during development (*P < 0.05, ANOVA). ED 18.5 baseline carotid arterial flow (B) of caffeine-treated embryos increased, whereas ED 18.5 dorsal aorta flow (D) decreased (†P < 0.05 vs. ED 18.5 sham group). Values are means ± SD. Experimental numbers were 24 mothers and 95 embryos in sham group and 25 mothers and 95 embryos in caffeine group, respectively.
tance may also explain some of the caffeine-induced regional variations in embryonic arterial blood flow (44). In the present study, caffeine-induced reduction in umbilical arterial blood flow peaked at ED 11.5, and the umbilical arterial blood flow became insensitive to caffeine dosing by ED 13.5. Relatively low placental resistance may preserve umbilical arterial blood flow despite increased embryonic vascular resistance, resulting in reduced embryonic carotid arterial blood flow (smaller vascular bed of developing brain vs. the placental vascular bed). An increase in placenta wet weight in the present study could support a part of this effect. Finally, caffeine may also affect the pharmacokinetics of adenosine within the developing embryo. Studies have shown that the plasma concentration of adenosine is usually higher in the fetus than in the adult (49) and that fetal adenosine levels increase under stress conditions such as hypoxia or ischemia (48, 49). Xu et al. (47) demonstrated that the adenosine A2A receptor agonist CGS-21680 increases the contractile amplitude in fetal chick ventricular cardiomyocytes. Thus a negative inotropic effect of caffeine on embryonic myocardium could occur via adenosine receptor blockade.

The underlying mechanisms by which maternal caffeine exposure induces intrauterine growth restriction remain unknown. Previous studies on high-dose caffeine exposure in developing embryos showed that higher doses of maternal caffeine exposure (30–120 mg·kg⁻¹·day⁻¹) decreases newborn offspring body weight and head and upper limb sizes (31). Tomimatsu et al. (42) recently reported that acute maternal caffeine exposure reduces fetal cerebral blood flow and local brain oxygen tension. These authors also described that local brain oxygen consumption increases without changes in circulating blood gas oxygen tension in near-term ewe fetuses (42). We found in the present study that with recurrent maternal caffeine exposure, the crown-rump length, upper limb size, and wet body weight of caffeine-treated ED 18.5 fetuses were smaller than sham fetuses, whereas the head size was unaffected. In addition, ED 18.5 caffeine-treated fetuses had increased carotid artery flow and decreased descending aorta flow, whereas umbilical artery flow was the same as in sham fetuses. We speculate that recurrent caffeine-treated fetuses preserve blood flow (oxygen and nutrient supplies) to the

![Fig. 4](image-url)  
**Fig. 4.** Time course changes in ED 11.5 embryonic CV function following a single dose of maternal caffeine exposure. Single-dose maternal caffeine exposure depressed the embryonic dorsal aorta flow, carotid artery flow, and embryonic ventricular contraction (LV-SF) (*P < 0.05 vs. baseline, ANOVA). The largest embryonic hemodynamic effects occurred at 30 min after maternal caffeine treatment. All hemodynamic data returned to baseline by 180 min. The numbers of recorded embryos are 21 embryos at baseline and 12 embryos at 90 and 180 min after caffeine administration. Values are mean ± SD.

![Fig. 5](image-url)  
**Fig. 5.** Maternal and embryonic hemodynamic effects at peak maternal serum caffeine concentration after daily caffeine treatment. Caffeine at peak maternal serum concentration did not change maternal HR, maternal CO, or embryonic HR during entire study stages (A–C, respectively). The ED 9.5 embryonic dorsal aorta flow (%DA; D), umbilical artery flow (%UA; E), and carotid artery flow (%CA; F) did not respond to caffeine dosing at peak maternal serum concentration, whereas these arterial flows significantly decreased at ED 11.5 (*P < 0.05 vs. ED 9.5 caffeine group; †P < 0.05 vs. baseline; ANOVA). Whereas dorsal aorta and umbilical artery flows became insensitive to the peak caffeine concentration at EDs 13.5 and 18.5, carotid artery flow remained decreased (†P < 0.05; F). Values are means ± SD. Experimental numbers of sham and caffeine mothers and embryos are the same as in Fig. 3.
weak adenosine A2A receptor gene expression in ED 9 mouse embryo. Although we could not detect regional adenosine A2A receptor blockade. Blackburn et al. (2) identified caffeine effects on embryonic CV function occurred via aden-
sosine A2A receptor mRNA was expressed within both the embryo-placen-
tal and uteroplacental systems. Changes in mRNA expression levels within embryo and uterus may reflect maternal caffeine effects on these local circulatory systems.

The affinity of adenosine A2A receptors for caffeine also plays an important role in determining caffeine-induced embryonic hemodynamic effects. Kull et al. (27) showed that human adenosine A2A receptor has a higher agonist affinity level than rat adenosine A2A receptor, whereas antagonist affinity level is similar between humans and rats. Species differences in adenosine sensitivity to caffeine may impact the embryonic hemodynamic response to caffeine.

Several limitations in the present study should be noted. First, the purpose of the current study was to investigate the maternal caffeine effect on developing embryonic CV function. We administered maternal caffeine commencing at ED 9.5, which corresponds to the onset of the heartbeat in the mouse embryo. In humans, maternal ingestion of caffeine before conception is likely, and therefore our protocol does not completely reproduce the human behavior of caffeine consumption. However, Pollard and colleagues (23, 34) reported that much higher caffeine doses do not influence conception and preimplantation embryo development in the rat animal model. They did report that caffeine affects postimplantation embryo development, which is consistent with our current results. Second, we did not investigate caffeine metabolites, such as paraxanthine, methylxanthine, and theophylline. These caffeine metabolites have pharmacological properties similar to caffeine and may have stronger antagonistic effects on adenosine receptors (7). In addition, recent studies have shown that cytochrome P-450-CYP1A2 enzyme activity, which is involved in caffeine metabolism, impacts fetal growth (19). Third, our data do not prove that blood flow redistribution in maternal caffeine-exposed embryos was due to the downregulation of adenosine A2A receptors. Further studies are required to determine whether the maternal caffeine effect on the blood flow distribution within fetoplacental circulation is related to the adenosine A2A receptor function. Finally, maternal caffeine effects in a mouse animal model may not reflect human effects. Therefore, our results of negative embryonic hemodynamic and growth effects of maternal caffeine exposure should be cautiously translated as to whether the results reflect humans.

In conclusion, the current study suggests that modest daily maternal caffeine exposure has a negative effect on embryonic CV function and overall embryonic growth, possibly mediated...
via adenosine A2A receptor blockade. These data do raise important questions that warrant further investigation to determine the maternal caffeine exposure threshold that impairs embryonic growth and fate and to confirm the underlying mechanisms.

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GRANTS

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