Prenatal nicotine exposure affects cardiovascular function and growth of the developing fetus

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論　文　内　容　要　旨

学位論文題名	Prenatal nicotine exposure affects cardiovascular function and growth of the developing fetus

背景：
ニコチンが胎児に様々な悪影響を及ぼすことは良く知られているが、ニコチンが妊娠初期の胎児の心血行動態に及ぼす影響は明らかではない。
目的：
妊娠初期のマウスにニコチンを投与し、母体および胎仔のエコー検査を施行することにより、胎仔が母体内で実際に起こしている心血行動態の変化を明らかにする。さらに出生後のマウスが成獣にいたるまでの発育と心機能を継続的に追跡調査することにより、妊娠中のニコチン投与が出生後に与える影響も検討する。
方法：
心臓形成時期にあたる妊娠日齢(GD)9.5、11.5、13.5 に、CD-1 妊娠マウスにニコチン 0.2mg/kg を皮下注射し、急性期の心血行動態の変化を対照群と比較して検討した。次にニコチン負荷の慢性的な影響を調べるために、GD6.5 から出産するまで 0.01%ニコチン水のみを摂取させた群と、普通水を摂取させた対照群に分けた。それぞれ GD9.5、11.5、13.5 にエコー検査を施行して検討し、両群から出生したマウスに対して発育と血圧、心機能等の追跡検査を成獣まで継続した。
結果：
妊娠マウスにニコチンを皮下注射した場合、母体の心機能には両群間に差は認めなかったが、胎児においては背側大動脈、総頚動脈および臍帯動脈の血流がそれぞれ減少する傾向があり、特に GD11.5 において有意に各血流が減少した。次にニコチン水を経口投与した場合にも母体の心機能には両群間に差を認めなかったが、胎児の各動脈血流においてはニコチン水摂取群の各血流が減少する傾向があり、特に GD13.5 では有意な血流減少を認めた。次に出生後の追跡調査では、ニコチン水摂取群からの出生仔は有意に低体重であり、その後成獣にあたる 9 週齢まで体重は小さなままで経過した。またニコチン群からの出生仔は出生後に心肥大と駆出率の上昇を認めたが、その他の心機能には両群間に明らかな差は認めなかった。
結論：
妊娠マウスへのニコチン投与により、急性期と慢性期のいずれにおいても胎仔の各臓器血流は減少することが判明した。胎盤を通過したニコチンが、その血管収縮作用により胎盤血流を減少させることにより胎仔の各臓器血流が減少し、胎児の組織低酸素状態をきたし、胎児発育不全や出生後の健康に影響する可能性が示唆された。

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ABSTRACT

Aim: Effects of nicotine on fetal hemodynamics are not well-known, especially in the first trimester fetus. We investigated the acute and chronic effects of nicotine on hemodynamics in pregnant mice and their fetuses using ultrasound. Postnatal health status including growth and hemodynamics was also examined.

Methods: To investigate the acute effects of nicotine on fetal hemodynamics, we injected nicotine 0.2 mg/kg subcutaneously into pregnant mice on gestational days (GD) 9.5, 11.5, and 13.5, and compared with saline-injected group. To determine the chronic effects of nicotine on fetal hemodynamics, we administered nicotine in drinking water (0.1mg/ml) to pregnant mice from GD 6.5 until they gave birth and compared hemodynamics with water administered mice.

Results: Regarding the acute effects of nicotine, we found no intergroup difference in maternal hemodynamics; however, fetal blood flow through the dorsal aorta, carotid artery, and umbilical artery tended to decrease, particularly on GD 11.5. Regarding the chronic effects of nicotine, we observed no intergroup difference in maternal body weight changes and hemodynamics; however, blood flow to all fetal organs tended to be lower in the nicotine water group than in the water group with significant difference on GD 13.5. The offspring of the nicotine water group had significantly low birth weights and continued to have low body weight until 9 weeks of age. In addition, these offspring developed postnatal cardiac hypertrophy.

Conclusion: Nicotine adversely affects fetal hemodynamics acutely and chronically in early pregnancy, potentially leading to fetal tissue hypoxia, intrauterine growth restriction, and adverse postnatal health effects.

Keywords: fetus, hemodynamics, neonate, nicotine

Abbreviations

GD	gestational days
VTI	velocity-time integral
DA	dorsal (descending) aorta
CA	carotid artery
UA	umbilical artery
FS	fractional shortening
mVcfc	rate-corrected mean velocity of circumferential fiber shortening
CO	cardiac output
LVPWs	left ventricular posterior wall thickness during systole
ESWS	end systolic wall stress
e'/a' ratio	e': peak early-diastolic velocity, a': peak late-diastolic velocity

1. INTRODUCTION

According to the results of a survey conducted by Japan's Ministry of Health, Labour, and Welfare, smoking rates among women in their 20s, 30s, and 40s in 2018 were 9.3%, 9.1%, and 12.6%, respectively. In a 2010 study performed in Japan, the smoking rate among pregnant women was reported as 5.0%, but the second-hand smoke exposure rate was 68.7%. Nicotine from maternal smoking is drawn into the placenta and amniotic fluid, passes into the fetus via the digestive system and cutaneous absorption, and accumulates in the fetus at concentrations 15% greater than that in the mother's body.[1] The effects of nicotine on fetal hemodynamics have been reported by studies in which experiments were conducted in sheep,[2,3] as well as by clinical studies on humans.[4] These studies were performed on pregnant subjects in the second trimester and later, however, no studies have examined the effects of nicotine on cardiac hemodynamics of a fetus at the early developmental stage. The objectives of the current study were to examine effects of nicotine on the hemodynamics of a first trimester-equivalent fetus *in vivo* and the postnatal hemodynamics in a minimally invasive way using a highly sensitive ultrasound imaging system for small animal.

2. METHODS

Animals

We used pregnant mice created by mating male and female CD-1 mice between 10 and 12 weeks of age (Charles river laboratories, Yokohama, Japan). The mating was carried out overnight using one male mouse for two to three nulliparous female mice. The following morning, if a vaginal plug was noted, gestational age was designated as gestational day (GD) 0.5 at noon. The study was performed according to the Fukushima Medical University

guidelines for animal experiments and was approved by its ethics committee for animal experiments (approved numbers: 24019, 26018 and 28025).

Evaluation of the acute response to nicotine administration

To examine the acute effects of maternal administration of nicotine on the maternal and fetal hemodynamics, we subcutaneously injected nicotine (MP Biomedicals, LLC, Solon, USA) 0.2 mg/kg (nicotine group, n=11) or normal saline (saline group, n=9) into the posterior necks of pregnant mice and evaluated their hemodynamic status. We adjusted the nicotine concentration to deliver a dosage of 0.01 ml/g of maternal body weight. We performed ultrasounds on the following 3 days: on GD 9.5, equivalent to approximately 20-22 weeks of human gestation, when formation of the heart loop begins; on GD 11.5, equivalent to approximately GD 27-30 of human gestation, when the heart septum is formed;[5] on GD 13.5, equivalent to approximately 40-42 weeks of human gestation, when the heart structure is nearly completely developed. In both groups we also performed ultrasounds prior to and 15-30 minutes post-injection to measure cardiac function and blood flow in various blood vessels of the mothers and fetuses.

Evaluation of the chronic response to nicotine administration

To investigate the chronic effects of maternal nicotine administration, we divided the pregnant mice into the nicotine water group (n=11) and water group (n=8). We administered nicotine in drinking water (0.1mg/ml) from GD 6.5 until they gave birth in the nicotine water group, and we administered plain drinking water in the water group. We performed ultrasound examinations in both groups on GDs 9.5, 11.5, and 13.5, and compared the hemodynamic changes in the mothers and the fetuses. After the mothers in both groups

delivered their pups, we tracked the postnatal growth of the offspring mice, comparing their weights, blood pressures, cardiac wall thicknesses, and cardiac functions until the ninth postnatal week.

Method of measuring maternal and fetal serum nicotine concentrations

Because it is technically difficult to obtain blood samples at different time points from the same animal, we prepared a separate group of animals for measuring serum nicotine concentrations. For the evaluation of the acute response to nicotine dosing, we injected nicotine 0.2 mg/kg into the posterior necks of GD 13.5 mothers and collected blood samples under deep anesthesia from their hearts at 5, 15, 30, and 60 minutes after the administration (each; n=2). We measured the serum nicotine concentrations using a HPLC. For the HPLC measurements, we used an ELITE LaChrom® (Hitachi High-Technologies Co., Japan), with Inertsil® ODS-3V (GL science, Inc., Japan) columns. For the evaluation of the chronic response to nicotine dosing, we collected blood samples from six mothers that were administered nicotine in drinking water (0.1mg/ml) over a 7-day period. To verify whether nicotine passes through placenta and accumulates in the fetus, we administered nicotine in drinking water (0.1mg/kg) from GD 6.5, and measured the average serum nicotine concentration of the fetuses that were removed from the mother at GD 18.5 when fetal blood can be collected. As before, HPLC was performed on the blood samples to measure the serum nicotine concentrations.

Ultrasound examination method

We used Vevo2100 (VisualSonics, Toronto, Canada), a high-sensitivity ultrasound imaging system for small animals. We administered general anesthesia with an inspired oxygen

concentration set at 21%, and isoflurane concentration set at 1.6–1.8% during the ultrasound examinations. We also used a heating table with temperature controller and a heating lamp to maintain the body temperatures of the mothers at approximately 37°C. We measured blood pressures (BP) of the mothers using BP-98AL® (Softron, Tokyo, Japan) which was a non-invasive tail cuff system. We used the M-mode to measure maternal ventricular wall motion, and pulsed-Doppler velocity tracings to measure maternal heart rate (HR) and velocity-time integral (VTI) of blood flow in the maternal pulmonary artery, fetal dorsal (descending) aorta (DA), fetal common carotid artery (CA), and umbilical artery (UA). As indicators of contractile function, we used fractional shortening (FS), and rate-corrected mean velocity of circumferential fiber shortening (mVcfc) of the left ventricle. We estimated maternal cardiac output (CO) and regional fetal arterial blood flow as VTI × HR (cm/min). As an indicator of ventricular hypertrophy, we used the left ventricular posterior wall thickness during systole (LVPWs); as an indicator of ventricular afterload, we used left ventricular end systolic wall stress (ESWS); as an indicator of left ventricular diastolic function, we used the e'/a' ratio (e': peak early-diastolic velocity, a': peak late-diastolic velocity), with tissue-Doppler of the mitral annulus. (Fig. 1). We measured the weights of the mice born to both the water and the nicotine water groups at the ages of 1 day, 1 week, 2 weeks, 3 weeks, 6 weeks, and 9 weeks. Their ultrasounds examination was performed at 3, 6, and 9 weeks of age under isoflurane inhalation anesthesia as with their mothers. Because it is difficult to discriminate between male and female after the age of 1 day until 3 weeks, this study was conducted regardless of gender.

Statistical methods

All measurements are shown as mean ± standard error (SE). As a result of the normality test,

our experimental data included data that was not normally distributed. Therefore, we performed the Mann-Whitney test to analyze differences between the two groups and set the significance level at <5% on either side of the distribution. For the analysis, we used SPSS version 22.0 for MacOS (IBM Japan, Ltd., Tokyo, Japan).

3. RESULTS

3.1 Acute responses

Maternal serum nicotine concentrations in the nicotine group

Measurements were taken from the blood samples collected from the mothers 5, 15, 30, and 60 minutes after nicotine injection. The serum nicotine concentration rose to its highest level (31.4 and 30.6 ng/ml) at 15 minutes post-injection, and then steadily decreased at the 30 and 60-minute measurements (Fig. 2).

Baseline levels of hemodynamics in the nicotine and the saline groups

Prior to the nicotine injections, there were no differences between the two groups with regard to maternal HR, FS, or pulmonary artery flow VTI x HR which represented CO. Regarding fetal blood flows to DA, CA and UA, there were no differences between the two groups, except for the UA flow on GD 9.5 (Fig. 3). On GD 9.5, UA blood flow VTI x HR was 45.8±11 cm in the saline group and 50.9±12 cm in the nicotine group (p=0.033).

Hemodynamic changes after nicotine administration in the nicotine and the saline groups

Figures 4 A–C show the percent changes in HR, FS, and CO among the mothers and Figures 4 D–F show the percentage changes in blood flow to the organs of the fetuses in the two groups. The HR, FS, and CO of the mothers rose in both groups on GDs 9.5, 11.5, and 13.5,

although there was no significant difference between the groups regarding the degree of increase (Figs. 4A–C). Systolic BP was also not significantly different between the two groups and before and after drug administration. Regarding blood flow to the fetal organs, we found a decreasing tendency in the DA, CA, and UA of the nicotine group (Figs. 4D–F). On GD 11.5 in particular, blood flow to the DA (−6.3±2.5%, $p<0.01$), CA (−8.6±2.8%, $p<0.01$) and UA (−19.6±4.9%, $p<0.01$) all decreased significantly in the nicotine group.

3.2 Chronic responses

Maternal and fetal serum nicotine concentrations and maternal body weight gain in the nicotine water and the water groups

One week after GD 6.5, the mean blood nicotine concentration following free oral intake of nicotine in drinking water (0.1mg/ml) was 19.5±8.9 ng/ml. The average serum nicotine concentration of the fetuses that were removed from GD 18.5 pregnant mouse was 77.4 ng/ml. From GD 6.5 to GD 13.5, there was an increase of 24.8% in the weights of the pregnant mice in the nicotine water group (n=17), from 38.1±2.2 g to 47.5±4.2 g. In the water group (n=20), on the other hand, there was an increase of 26.6% in the body weights, from 35.4±2.6 g on GD 6.5 to 44.7±3.5 g on GD 13.5. We did not observe a significant difference between the groups ($p=0.51$).

Comparison of the hemodynamics of the pregnant mice and fetuses in the nicotine water and the water groups

When we compared the HR, FS, and CO of the mothers of both groups on GDs 9.5, 11.5, and 13.5, there were no significant differences (Figs. 5A–C). Maternal systolic BP in the nicotine water group on GDs 9.5, 11.5, and 13.5 were 84.5±6.5, 82.1±5.8, and 88.1±3.8

mmHg, respectively, and that in the water group on GDs 9.5, 11.5, and 13.5 were 85.2±5.3, 87.0±5.8, and 87.8±3.8 mmHg, respectively. The BP was not significantly different between the two groups and among each GD. With regard to fetal hemodynamics, the blood flow to all the DA, CA, and UA in the nicotine water group tended to be lower than in the water group. On GD 13.5, in particular, the blood flow to DA (246.7±7.5 cm, $p=0.025$), CA (144.1±4.4 cm, $p=0.020$) and UA (170.2±5.4 cm, $p<0.01$) were significantly lower in the nicotine water group. (Figs. 5D–F).

Results of tracking the growth and hemodynamic status of mice born to the nicotine water and the water groups

The number of births per one pregnant mouse in the water group and nicotine water group were 11.3±1.6 and 12.5±1.3, respectively, and there was no significant difference between the two groups. We studied the growth and cardiac function of the offspring by tracking them until maturity. Compared to the water group, the mice born to the nicotine water group showed significantly lower weights from immediately after birth until maturity at 9 weeks of age (Fig. 6A). We found no significant difference between the groups with regard to systolic BP or HR (Figs. 6B–C). The nicotine water group had significantly higher LVPWs than the water group at 3 and 6 weeks of age, but by the time they reached maturity at 9 weeks of age, there was no significant difference between the groups (Fig. 7A). The FS was significantly higher in the nicotine water group than in the water group at 3 and 6 weeks of age, but at 9 weeks of age the FS levels were similar in the two groups (Fig. 7B). No significant differences were observed between the groups regarding the mVcfc, the e'/a' ratio, left ventricular ESWS, or CO (Figs. 7C–F).

4. DISCUSSION

Smoking during pregnancy leads to fetal growth restriction (FGR), premature birth, fetal death, neonatal birth defects and brain disorders, which have been demonstrated in a large number of studies.[6-8] By inhibiting the production of prostacyclin and directly constricting blood vessels via α receptors, nicotine decreases uteroplacental blood flow and the supply of oxygen to the fetus.[2] In addition, because nicotine accumulates in the fetus at concentrations 15% greater than that in the mother's body,[1] nicotine may directly affect the fetus.

The effects of nicotine on fetal heart hemodynamics have been conducted in studies using large animals and humans, but these studies are limited to the second trimester and later.[2,3] The reason is that the effects on fetal circulation at the early developmental stage has only been studied using chicken embryos or fetal hearts that were removed from the uterine environment. In order to investigate the true effects of maternal nicotine administration on the fetus, we believe that the optimal study should be minimally invasive and performed *in utero*. The lack of an experimental setup of this kind may be the reason why little progress has been made regarding research on the first trimester fetal circulation. Previously, we investigated the cardiac function of mouse fetuses during the period of organogenesis (the first trimester), using a highly sensitive ultrasound imaging system for small animals (Vevo 660, VisualSonics, Toronto, Canada).[9] This experimental setup allowed us to measure the hemodynamics of a first trimester-equivalent fetus *in vivo* and in a minimally invasive way.

Epidemiological research and animal experiments have shown the relationship between fetal nicotine exposure and obesity, hypertension, impaired glucose tolerance, and hyperlipidemia that extends beyond childhood, which suggests a link to fetal programming.[7,10-15] In the fetal programming theory, if a fetus is exposed to stressors *in utero,* such as drugs or a poor nutritional state, the fetus tries to conform to a "thrifty phenotype" by making circulatory

adjustments, energy metabolism adjustments, and endocrine adjustments. When a “thrifty phenotype” persists postnatally, it overcompensates for life in the extrauterine environment, leading to the development of lifestyle-related diseases such as obesity, impaired glucose tolerance, heart disease, and hypertension.[16] In considering the effects of nicotine on the fetal programing, it is important to examine the effects of nicotine on fetal hemodynamics during organogenesis.

In the current study, by using ultrasound to observe hemodynamic changes in mothers and fetuses, we were able to assess the effects of nicotine on the fetal circulation *in vivo*, as well as investigate its effects on health status, including postnatal growth and cardiac function. First, we observed acute-phase hemodynamic changes that occurred in the mothers and fetuses immediately after nicotine administration. The blood nicotine concentrations of mothers injected with nicotine 0.2 mg/kg peaked at 31.0±0.57 ng/ml, 15 minutes post-injection. When humans smoke, their peak nicotine blood concentration is generally considered to be 20–50 ng/ml; thus, we believe that the nicotine dose in the current study was appropriate.[16,17] Nicotine acts by stimulating the sympathetic ganglia, releasing norepinephrine, and causing the adrenal medulla to release epinephrine and norepinephrine. It also stimulates chemoreceptors in the aortic and carotid bodies, reflexively increasing HR, causing vasoconstriction, and raising BP.[18-20] However, at a dose of 0.2 mg/kg, we did not find any significant difference in the changes of maternal HR, BP, FS or CO, between the nicotine and saline groups. In other words, the injected nicotine 0.2 mg/kg may not have been high enough to affect hemodynamics in the mothers. In contrast, we did find a significant difference between the nicotine and saline groups in blood flow to the fetal organs. Guan et al. reported that when a low dose of nicotine was administered intravenously into second trimester-equivalent ewes, maternal BP and HR did not change, but the fetal

HRs were lowered.[21] Suzuki et al. reported that when nicotine was intravenously injected into third trimester-equivalent rhesus monkeys, the fetus' uterine artery blood flows decreased, and hypoxemia and acidosis were observed.[22,23] Clark et al. reported that when nicotine was administered intravenously to third trimester-equivalent ewes, fetal BP rose significantly, but fetal HR and umbilical blood flow decreased.[3] These reports have shown that nicotine adversely affects fetal hemodynamics, which is consistent with the results of the current study. However, while all of those previous reports have focused on second through third trimester-equivalents, our study focused on the first trimester, which is the time of cardiogenesis. Therefore, to the best of our knowledge, the present study is the first to report the acute effects of nicotine on fetal hemodynamics in early pregnancy.

Next, we investigated the chronic effects of nicotine on fetuses using a group of pregnant mice that were allowed free oral access to nicotine water only. We measured their serum nicotine concentrations at GD 13.5 and found a mean concentration of 19.5±8.9 ng/ml. Comparing this with the previously-mentioned peak blood concentration (20–50 ng/ml) related to human smoking, the dosage seems to have been appropriate. Regarding the chronic effects of nicotine, there was no significant difference in the HR, BP, FS, or CO of the mothers, however, blood flow to the DA, CA, and UA tended to be lower in the nicotine water group; at GD 13.5 in particular, all blood flow parameters were significantly lower in the nicotine water group. MacArthur, et al., Prabhu, et al., and Suzuki, et al. all reported that if smoking is continued into the second or third trimester of pregnancy, the fetus and neonates will be small; however, if smoking is discontinued during the first trimester of pregnancy, there will be no differences in body build between fetuses and neonates born to women who quit smoking and those born to non-smoking women.[23-25] In contrast, the present study shows that adverse effects of nicotine also occur in the fetus during early pregnancy. Additionally, since a significant

decrease in blood flow was observed on GD 13.5, the effect of nicotine on the fetus was considered to be cumulative. There are two main possibilities for the mechanism of nicotine adverse effects on the fetus. First, nicotine constricts the uterine artery, causing fetal hypoxia and reducing the fetal heart function. Second, since it was verified in the present study that nicotine passed through the placenta and accumulated in the fetus, nicotine induces vasoconstriction of the fetus, resulting in the increase in preload and cardiac dysfunction. Further investigations on the effects of nicotine, such as uterine artery blood flow, fetal blood pressure, and fetal oxygen saturation, are necessary to determine the mechanism.

We continued nicotine water administration until the mice gave birth and compared the growth and cardiac function of the offspring with that of the mice born to the water group. From birth onward, the offspring born to the nicotine water group were significantly smaller compared to those in the water group until 9 weeks of age. This result is consistent with previous reports that smoking during pregnancy results fetal growth restriciton.[1,4,6-8,26] The reason why the offsprings born to the nicotine water group remained consistently small until 9 weeks of age was probably because the blood concentration of nicotine in the fetus was 77.4 ng/ml, which was considerably high.

Because ultrasounds and BP measurements from birth to 2 weeks of age would have been technically challenging due to the small body size of the offspring, we performed follow-up evaluation of cardiac function at 3 weeks, the earliest time point for ultrasound, which was performed again at 6 and 9 weeks of age. Although there was no significant difference in the offspring's HRs and BPs until 9 weeks of age between the groups, we found significant left ventricular hypertrophy in the nicotine water group at 3 and 6 weeks of age compared to the water group; however, by 9 weeks of age this abnormality had disappeared. Similarly, we observed higher FS in the nicotine water group at 3 and 6 weeks of age. There have been many

reports on an association between smoking of mothers in pregnancy and higher blood pressure in their offspring of various ages, both children and adults. However, there have been few reports on the relationship between maternal smoking and their offspring neonatal blood pressure. Furthermore, there have been no reports on the effects of smoking in pregnant mothers on their offspring's neonatal cardiac function. Geerts et al. showed that tobacco exposure in fetal life is independently related to an increase in systolic blood pressure in the early postnatal period.[27] Beratis et al. reported that neonates and infants of mothers who smoked during pregnancy have an elevation of BP that was related to the number of cigarettes smoked per day, but the BP returned to normal during the second year of life.[28] The inability to measure BP in fetuses and early postnatal mice makes detailed investigation difficult, but it is speculated that increased cardiac afterload caused by high nicotine concentrations stressed the cardiomyocytes in the fetuses and early postnatal mice. In addition, there is a possibility that myocardial hypertrophy and transient increases in cardiac FS occurred as a reaction to this stress.

5. LIMITATIONS

Because of the technical difficulty of assessing uterine artery blood flow in the CD-1 female mice, it is difficult to determine whether the responses we observed were due to uteroplacental ischemia in the pregnant mice, the effects of nicotine as it passed through the placenta to the fetus, or both. In addition, it should be considered including gender differences in postnatal examinations. However, we could not consider gender differences because we weighed mice right after birth, when it is difficult to determine gender. Finally, these ultrasound examinations were performed by almost one author, so inter-rater reliability was not examined, but sufficient pilot studies were repeated until reliable data were obtained.

6. CONCLUDING REMARKS

We found that by administering a nicotine dose to mice in the first trimester-equivalent of pregnancy, fetuses responded with an immediate, acute-phase decreases in blood flow to the DA, CA, and UA, and that this response was particularly significant on GD 11.5. Blood flow to the DA, CA, and UA also tended to decrease as a chronic response to orally administered nicotine water, and the blood flow to each decreased significantly on GD 13.5 in particular. Moreover, mice born to the nicotine-dose group were found to have FGR, with significantly lower body weights; however, this may have been due to decreased placental blood flow because of the vasoconstrictive action of nicotine. Nicotine during pregnancy is capable of causing not only low postnatal body weights, but also decreased blood flow to all fetal organs, which suggests that nicotine exposure may play a large part in the postnatal health.

7. DISCLOSURES

The authors have no conflicts of interest directly relevant to the content of this article.

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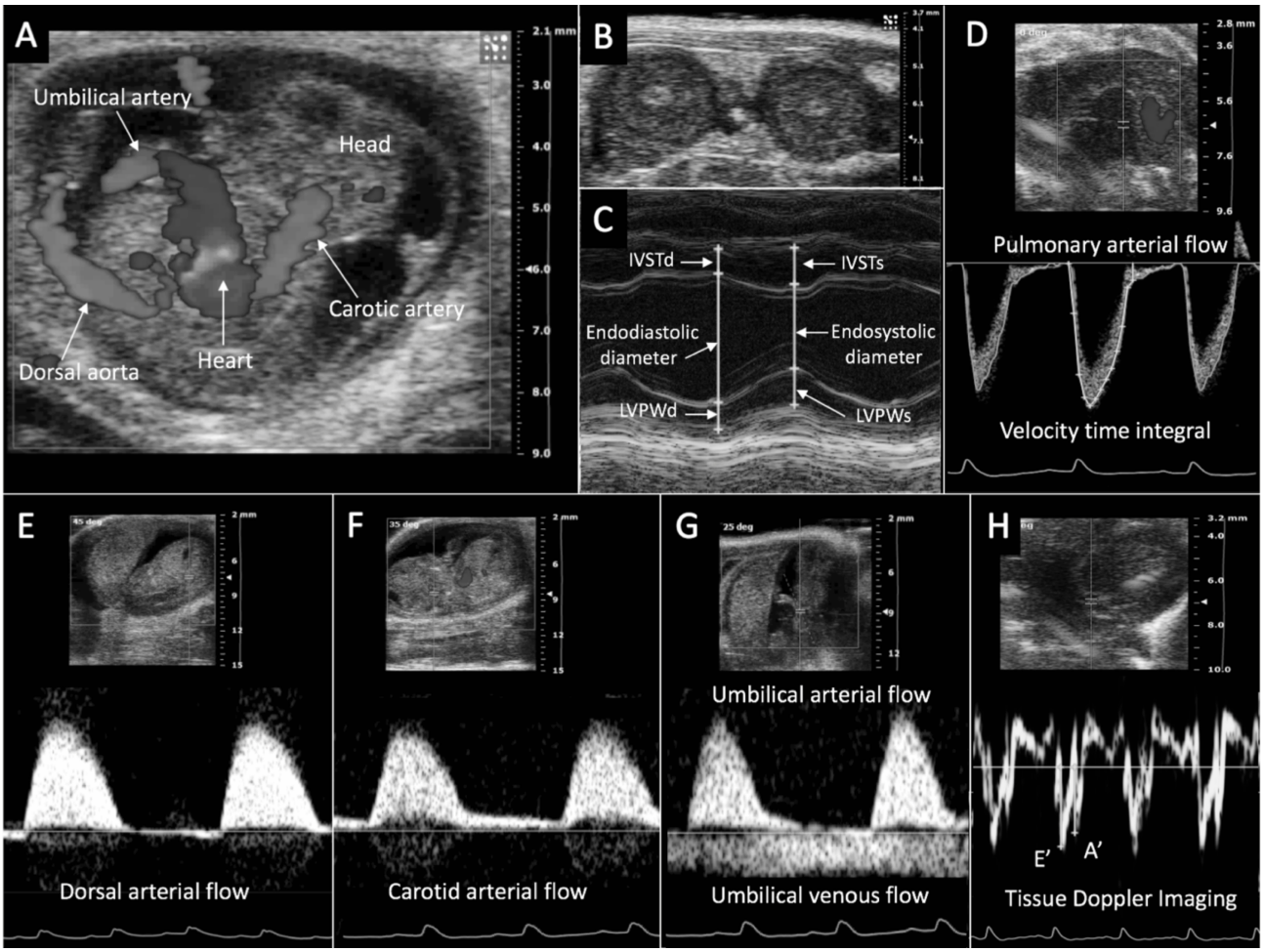

Figure1.
Ultrasound images of mothers and fetuses.
A: Color Doppler ultrasound images of the fetus on gestational day (GD) 11.5.
B: Embryo on GD 6.5 (fertilized eggs).
C: Left ventricle “M-mode” images of the mother.
D: Pulmonary arterial flow and VTI (velocity time integral) in the mother.
E: Dorsal aortic blood flow (DA) in the fetus on GD 11.5.
F: Common carotid arterial flow (CA) in the fetus on GD 11.5.
G: Umbilical arterial flow (UA) and umbilical venous flow (UV) in the fetus on GD 11.5.
H: Tissue Doppler imaging at the mitral annulus of the mother on GD 11.5.

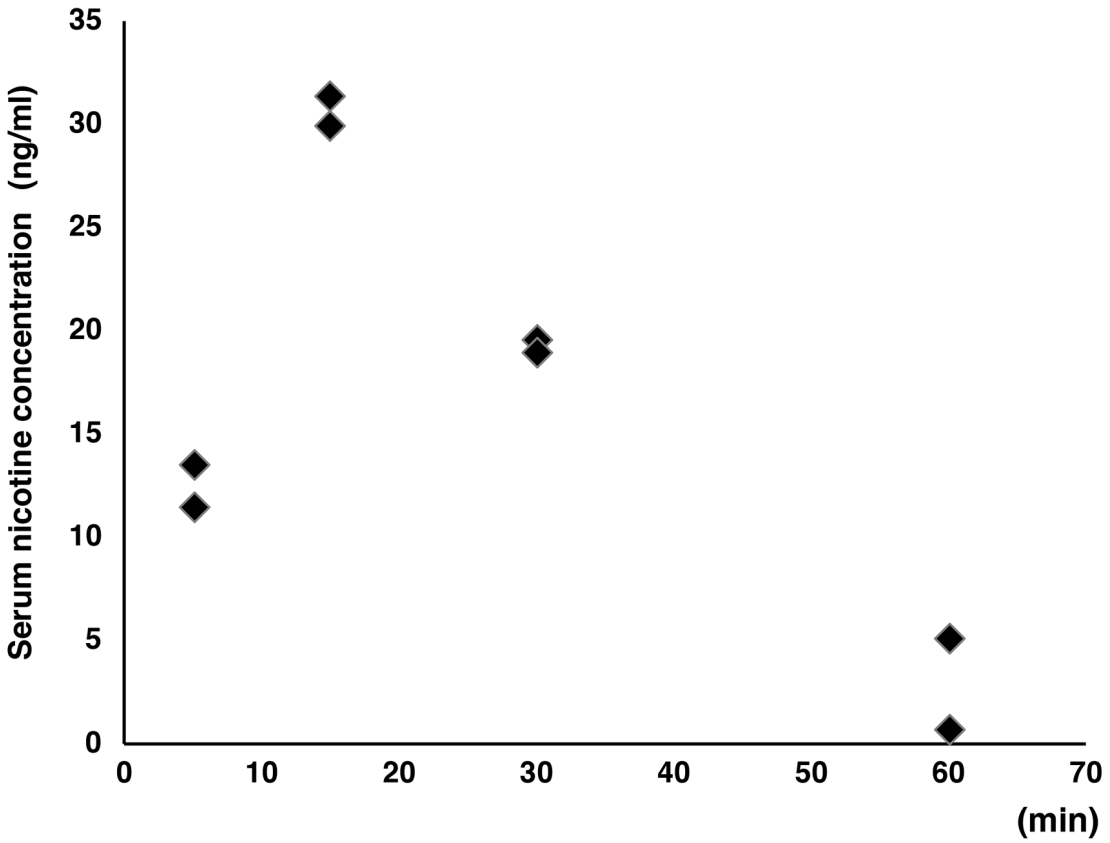

Figure 2.
Changes in serum concentrations of nicotine injected subcutaneously in the mother.
Nicotine serum concentrations at 5, 15, 30, and 60 minutes after subcutaneous injections of nicotine 0.2 mg/kg into the posterior neck of GD 13.5 mothers.

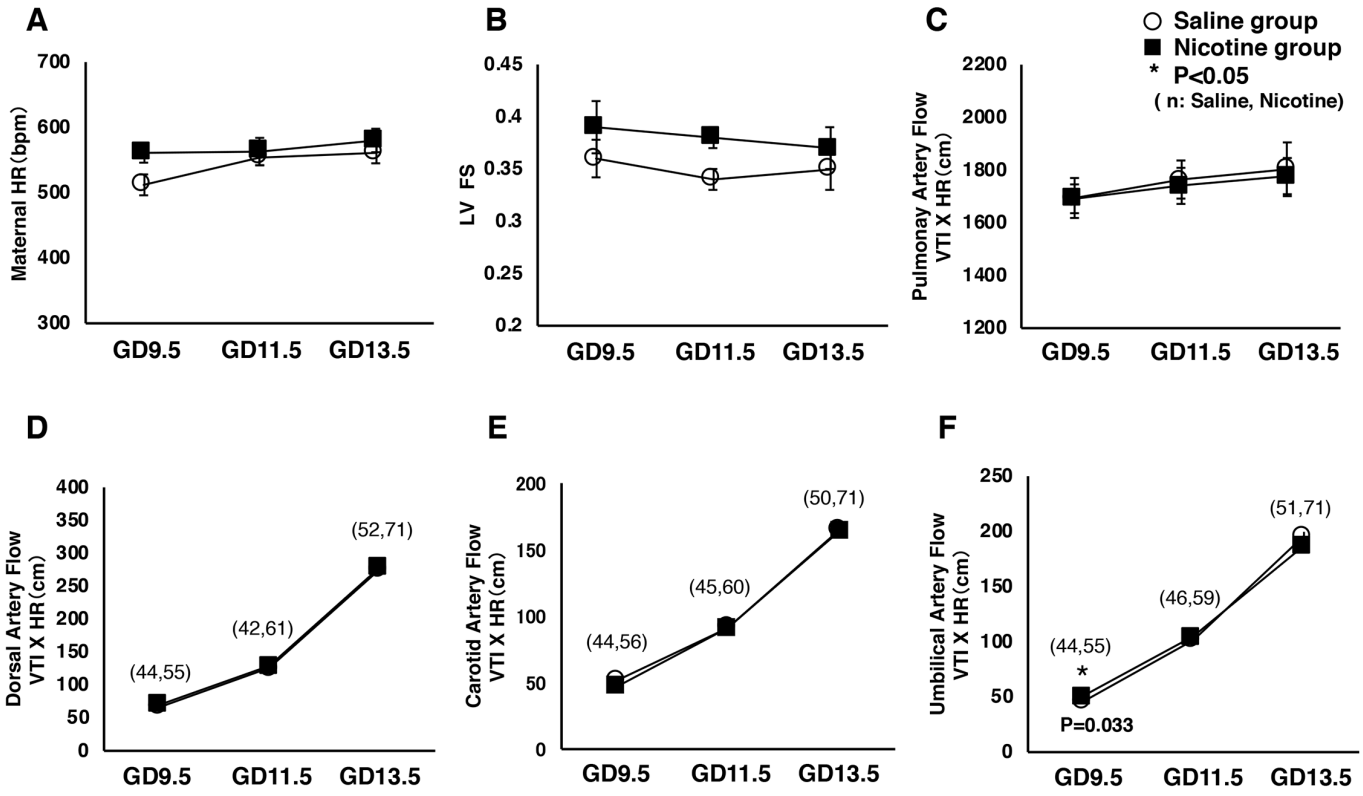

Figure 3.
Cardiac function in mothers and blood flow to the organs of fetuses in both nicotine and saline group prior to subcutaneous injections of either nicotine or saline.
A: Heart rate (HR) of mothers.
B: Left ventricular (LV) fractional shortening (FS) of mothers.
C: Pulmonary artery (PA) flow velocity-time integral (VTI) x HR, which was used to represent cardiac output of mothers.
D: Dorsal aortic (DA) blood flow VTI x HR of fetuses.
E: Common carotid arterial (CA) blood flow VTI x HR of fetuses.
F: Umbilical arterial (UA) blood flow VTI x HR of fetuses.
Values are presented as means ± SE.
Experimental numbers of mothers in saline group and nicotine group were 9 and 11, respectively.
Experimental numbers of fetuses are shown in parentheses as (saline group number, nicotine group number).

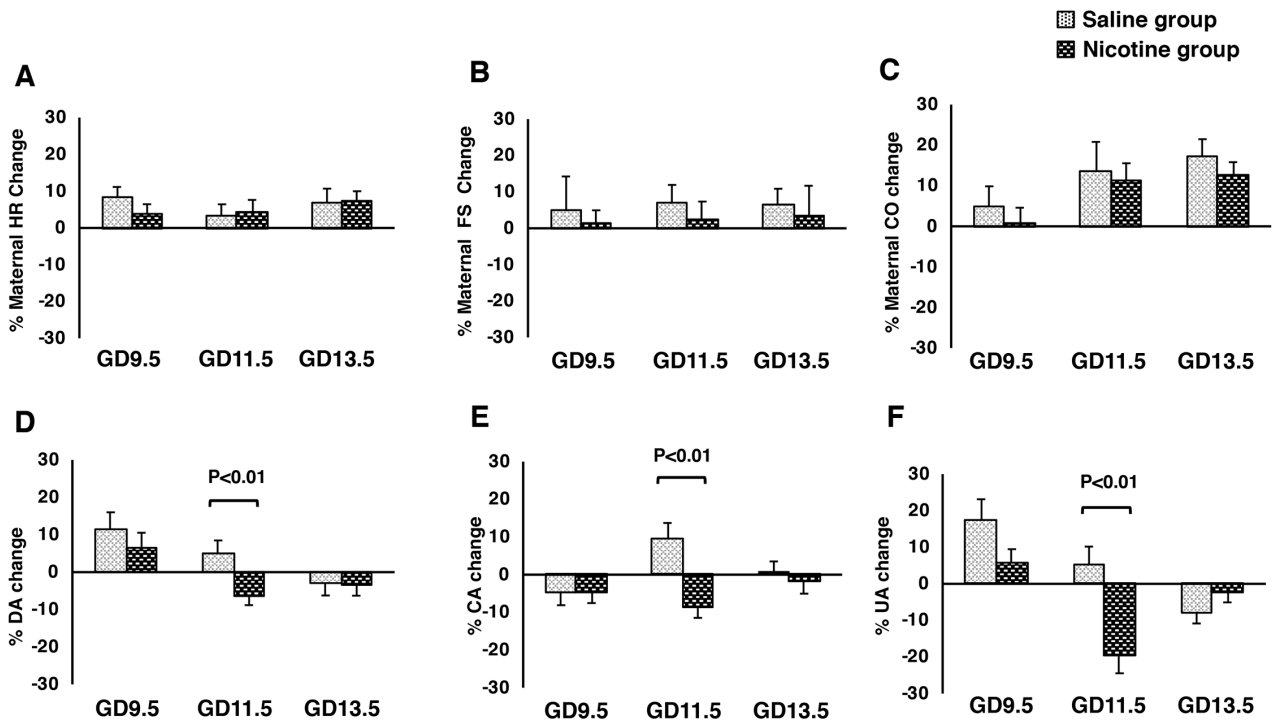

Figure 4.
Percentage changes in cardiac function of mothers and percent changes in blood flow to the fetal organs before and after administration of either nicotine or saline (acute response to nicotine).
A: Heart rates (HR) in mothers.
B: Cardiac fractional shortening (FS) in mothers.
C: Cardiac outputs (CO) in mothers.
D: Dorsal aortic (DA) blood flow of fetuses.
E: Common carotid arterial (CA) flow of fetuses.
F: Umbilical arterial (UA) blood flow of fetuses. Experimental numbers of mothers and fetuses in saline and nicotine group are the same as in Figure 3.
Values are presented as means ± SE

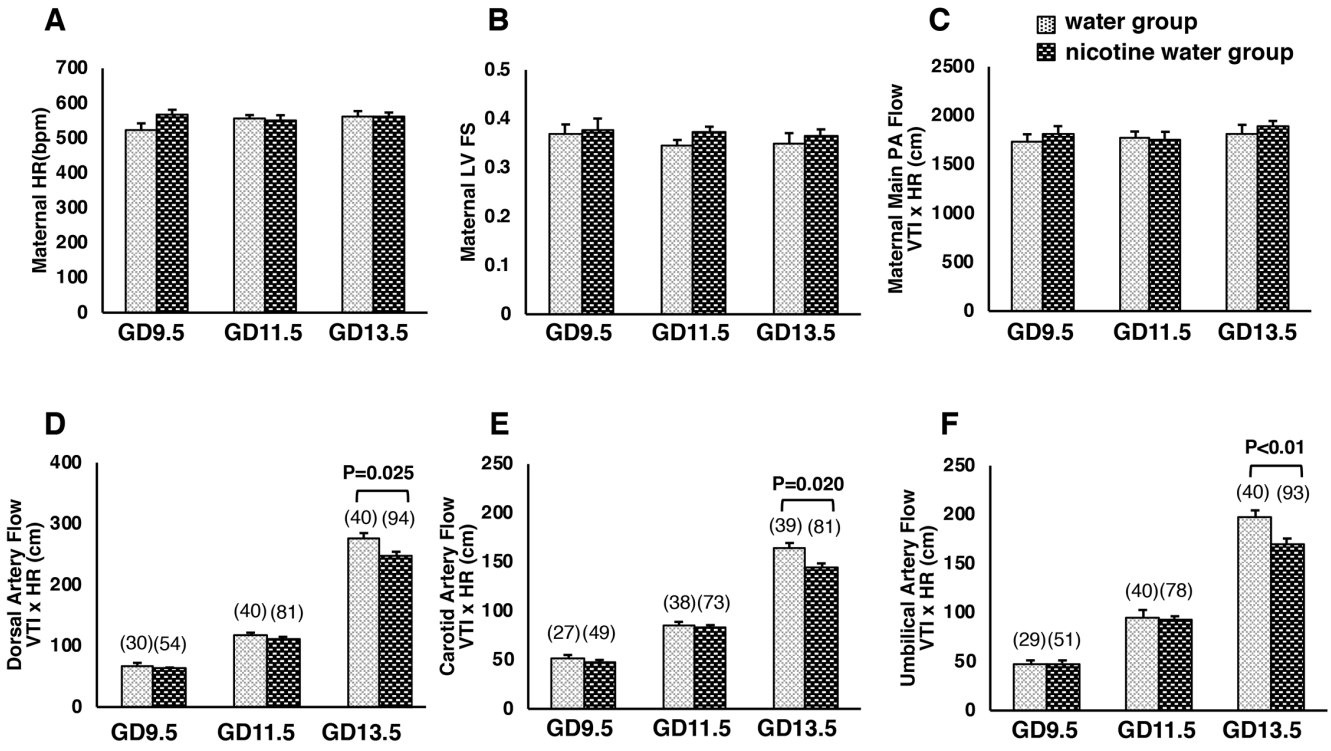

Figure 5.
Cardiac function in mothers and blood flow to fetal organs in the nicotine water and the water groups (chronic response to nicotine).
A: Heart rates (HR) in mothers.
B: Left ventricular shortening (FS) in mothers.
C: Pulmonary artery flow VTI x HR was used to represent CO of mothers.
D: Dorsal aortic (DA) blood flow of fetuses.
E: Common carotid arterial (CA) flow of fetuses.
F: Umbilical arterial (UA) blood flow of fetuses.
Experimental numbers of mothers in water group and nicotine water group were 8 and 11, respectively.
Experimental numbers of fetuses are shown in the parentheses.
Values are presented as means ± SE

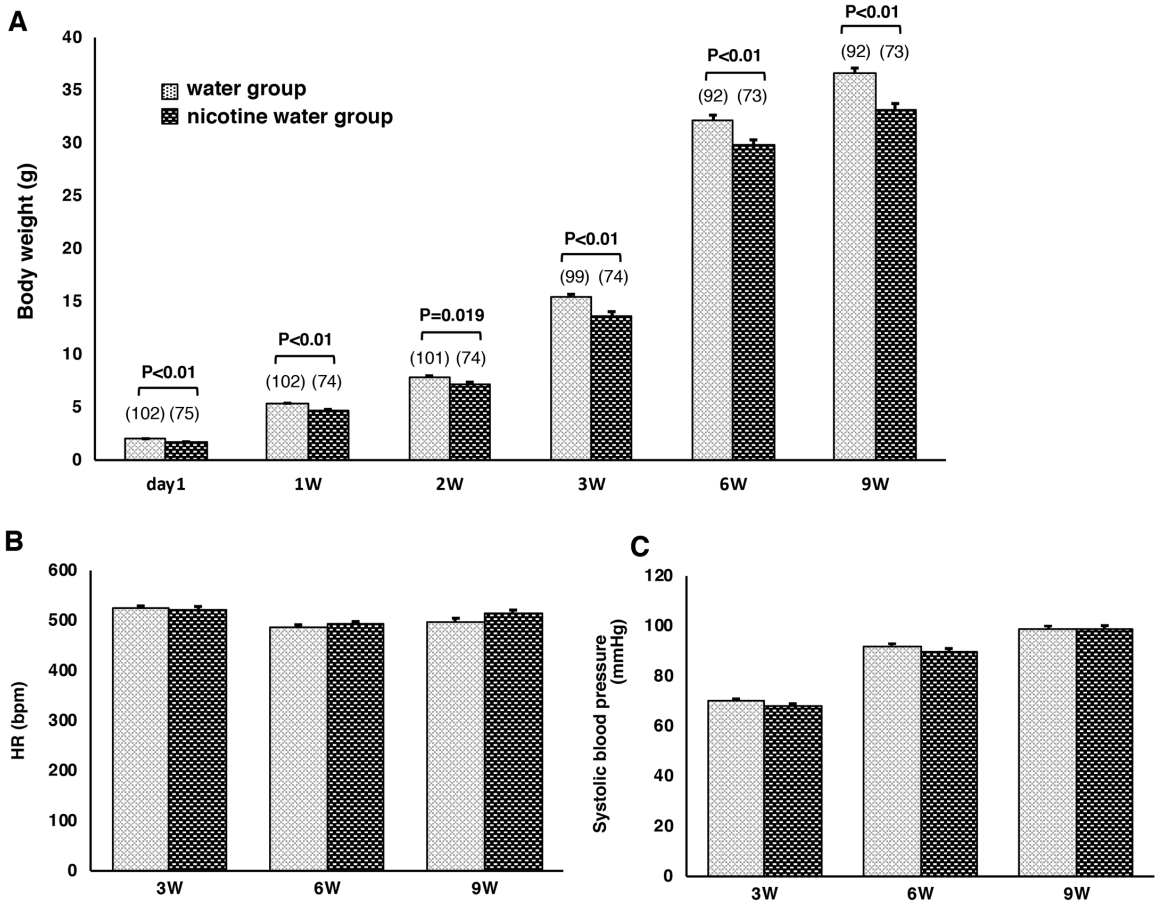

Figure 6.
Body weight, heart rate (HR), and systolic blood pressure of mice born to mothers that consumed either nicotine water or normal water.
A: Postnatal changes in mice body weight.
B: Postnatal changes in heart rate (HR)
C: Postnatal changes in systolic blood pressure. Experimental numbers of mice are shown in the parentheses.
Values are presented as means ± SE

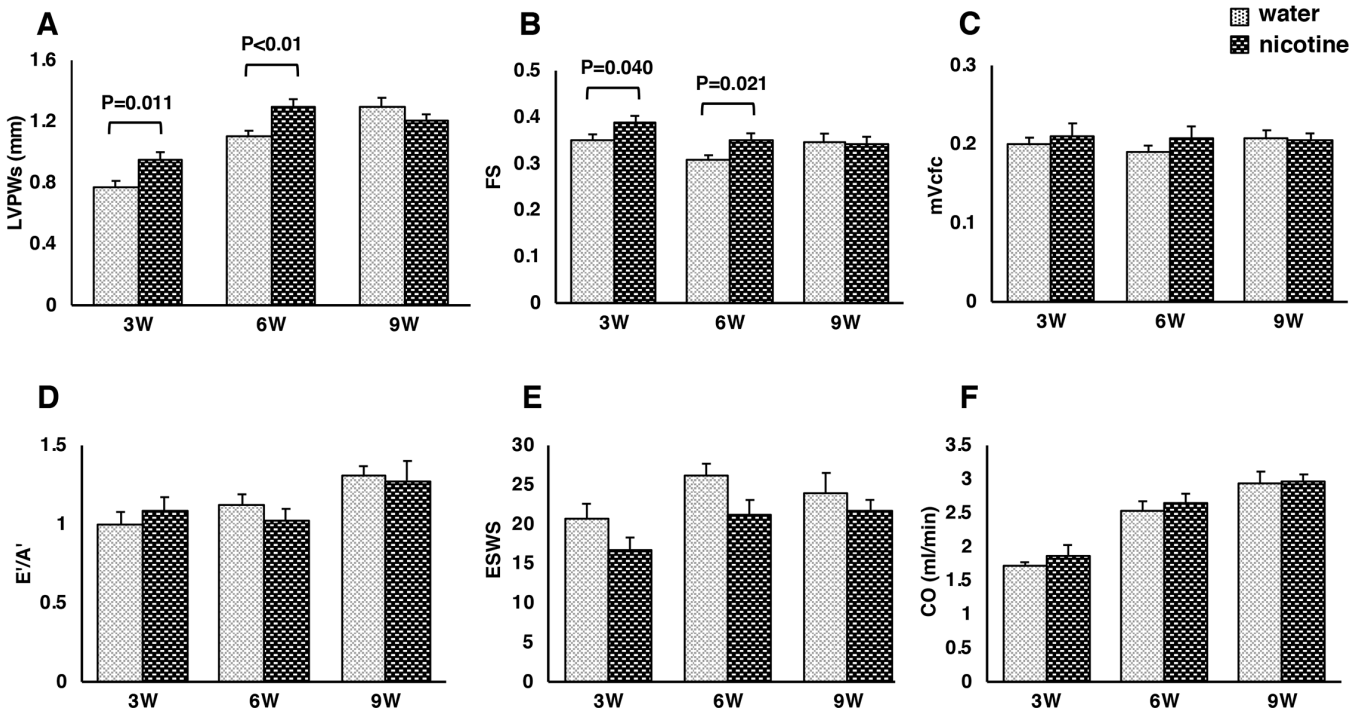

Figure 7.
Cardiac function in mice born to mothers that consumed either nicotine water or normal water.
A: Left ventricular posterior wall thickness in systole (LVPWs).
B: Left ventricular fractional shortening (FS).
C: Left ventricular rate-corrected mean velocity of circumferential fiber shortening (mVcfc).
D: E'/A' ratio of mitral flow.
E: Left ventricular end systolic wall stress (ESWS).
F: Cardiac output (CO).
Experimental numbers of mice in both groups are the same as in Figure 6.
Values are presented as means ± SE