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Orginal Research Article

Effects of Maternal Nicotine Exposure during Pregnancy and Lactation on Blood Pressure of the Offspring and Blood Vessel Structure and Attenuation by Vitamin C

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Abstract: **Background:** Recent epidemiological studies have shown that there is an increased risk of hypertension in children born to women who smoked during pregnancy. **Objective:** The aim of this study was to examine the effect of fetal and neonatal exposure to nicotine, the major addictive component of cigarette smoke, on blood pressure of offspring and blood vessel structure, as well as if this hypertension induced by maternal nicotine exposure can be prevented or attenuated by vitamin C supplementation during the perinatal period or after weaning. **Material and Methods:** Female Wistar rats were given nicotine (1 mg/kg/day in 1 ml s.c), either saline (1 ml/day, s.c) and nicotine (1mg/kg/day in 1 ml, s.c) + vitamin C (1g/l in the drinking water) during pregnancy and lactation. It was also animals group that received vitamin C (1g/l) in the drinking water from weaning up to 5 months of age. Blood pressure was determined in the female and male offspring from weaning until 20 weeks of age. At the end of the experimental period (20 weeks), the wall structure of the abdominal aorta in was examined. **Results:** Maternal nicotine exposure during gestation and lactation resulted in an increased blood pressure of male offspring but not of female offspring. The result of this study also indicates that blood pressure in male offspring increased from the age of 12 weeks onwards for the male offspring of nicotine-exposed animals. Maternal vitamin C supplementation in rats exposed to nicotine during pregnancy and lactation did not prevent development of hypertension of the male offspring, whereas the vitamin C given to pups after weaning significantly improved the nicotine induced hypertension of male offspring. The wall structure of the abdominal aorta of the nicotine group was irregular and abnormal alignment of three tunics, whereas this effect was not observed in the abdominal aortic from the offspring of the saline group. **Conclusion:** Our study demonstrated that maternal nicotine exposure during gestation and lactation increased blood pressure of male offspring but not of female offspring and caused an alteration in the wall structure of the abdominal aorta, thus providing insight into the mechanisms underlying the increased prevalence hypertension in children exposed to cigarette smoke in utero. Also, the supplementation with vitamin C after weaning leads to a reduction of the hypertension. To our knowledge this is the first time that it is shown that hypertension induced by nicotine exposure during pregnancy and lactation, can be attenuated or even reversed. It will also be of interest to determine whether epigenetic changes occurred.

Keywords: Maternal nicotine exposure, neonatal nicotine exposure, hypertension of the offspring, Wistar rats, vitamin C attenuated hypertension of the offspring.

1. INTRODUCTION

Reports from epidemiological studies suggest that exposure to nicotine is associated with the development of some chronic diseases such as, hypertension, obesity and type 2 diabetes (Gao, Y.-J. *et al.*, 2008). It has been reported that blood pressure can be increased during the smoking, due to the presence of nicotine in tobacco smoke (Xiao, D. *et al.*, 2014). This because the nicotine affects the cardiovascular system in two ways: 1) it increases heart rate and 2) it causes narrowing of arteries which increases to the risk of developing peripheral artery disease (PAD), affecting the arteries that supply the kidneys, stomach, arms, legs and feet, and also leads to increases in blood pressure.

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It is becoming increasingly clear that fetal exposure to nicotine has numerous consequences that is to the detriment of the health of the fetus, and that these effects may last well into adulthood. In pregnant women who smoke or use nicotine replacement therapy (NRT), nicotine crosses the placenta, concentrates in fetal blood and amniotic fluid, and is detectable in breast milk during lactation (Mızrak, S. *et al.,* 2012). Studies have shown that exposure to maternal nicotine is associated with an increased risk of elevated blood pressure in postnatal life. Xiao *et al.,*. (2008) reported that, in utero exposure to maternal nicotine (6 mg/kg/day) had no effect on baseline BP but significantly increased Ang II stimulated BP in male but not female offspring. On the other hand, Gao *et al.*, (2008) has showed that blood pressure was increased at 14 weeks of age in the nicotine (1 mg/kg/day) exposed male offspring.

In adults, it was shown that the cardio-ankle vascular index (CAVI) was increased by exposure to nicotine. The CAVI reflects the stiffness of some arteries in the body such as the aorta, femoral artery, and tibial artery (Noike, H. *et al.,* 2010). The cardio-ankle vascular index has suggested that the CAVI is a clinical marker for evaluation of atherosclerosis and arteriosclerosis in patient with essential hypertension (Okura, T. *et al.,* 2007). In hypertension patient's aortic stiffness is an independent predictor of cardiovascular mortality, fatal and non-fatal coronary events, and fatal strokes (Laurent, S. *et al.,* 2001). Nicotine is believed to alter the structural and functional characteristics of vascular smooth muscle and endothelial cells by enhancing endothelial proliferation and increased atherosclerotic plaque formation. Neovascularisation stimulated by nicotine has also been suggested to help the progression of atherosclerotic plaque. These effects lead to myointimal thickening, atherogenic and ischemic changes that increase the incidence of hypertension (Mishra, A. *et al.,* 2015). It has been reported that atherosclerosis and related diseases are due to vascular disorders including endothelial dysfunction (Rajendran, P. *et al.,* 2013). It has also suggested that nicotine has an effect on the regulation of vascular tone through alteration in per vascular adipose tissue (PVAT) composition and modulator function (Xiao, D. *et al.,* 2008), and this mechanism is associated with an increase in blood pressure (Gao, Y.-J. *et al.,* 2008).

Previous studies have reported that the harmful effects of nicotine is due to a disturbance in the capacities of endogenous antioxidant defenses in which the activity of the antioxidant system is overwhelmed by reactive oxygen species generation (Halliwell, B., & Whiteman, M. 2004). Oxidative stress due to nicotine exposure has been reported in various tissues, including lung, vasculature and pancreas. It had been suggested to be involved in the pathogenesis of hypertension and atherosclerosis (Montezano, A. C. *et al.,* 2015). Laboratory animal studies have established that angiotensin II (ANG II)-induced hypertension in the rat is accompanied by oxidative stress in blood vessels (Ocaranza, M. P. *et al.,* 2014). Xiao *et al.,* (2011) reported antenatal nicotine exposure to increase the risk of hypertension in adult offspring as a result of programmed heightened oxidative stress and vascular reactivity via a Nox2-dependent mechanism. It has been suggested that the toxicity of nicotine induced oxidative stress can be modulated by antioxidants (McIntyre, M. *et al.,* 1999).

As an antioxidant, vitamin C helps in preventing oxidative stress by directly scavenging oxygen-derived free radicals, such as superoxide anions or hydroxyl radicals (Nagaraj, S. K. D., & Paunipagar, P. V. 2014). It has been demonstrated that vitamin C protects nitric oxide (NO) from oxidation and increases its synthesis, and by this protection, it increases endothelial NO and ameliorates endothelial dysfunction (Taddei, S. *et al.,* 1998). Evidence also indicates that vitamin C is beneficial to healthy subjects and those with cardiovascular disease (CVD) (Brown, A. A., & Hu, F. B. 2001).

Therefore, the goal of this study was to examine the effect of nicotine exposure during pregnancy and lactation on the hypertension and the aorta morphology of the offspring. It was also to determine whether hypertension can be prevented or attenuated by vitamin C supplementation during the prenatal period or after weaning.

2. MATERIALS AND METHODS

Only animals free from visible signs of diseases and ill health were used in this study. Animals had free access to food (chow) and tap water. The rats were kept in a room with controlled temperature (23C°), controlled lighting (lights on at 7 a.m. and off at 7 p.m.) and controlled humidity.

Female Wistar rats were given either saline (1 ml/day, s.c; control group), or nicotine (1 mg/kg/day in 1 ml s.c; the nicotine group), during pregnancy and lactation. Blood pressure of animals was measured every two weeks from 5 weeks of age. Systolic blood pressure (SBP), diastolic blood pressure (DBP), mean arterial pressure (MAP) and heart rate (HR) were measured in rats by the tail-cuff method with a computerized blood pressure monitor (CODA blood pressure monitor, Kent Scientific, USA). The occlusion cuff was placed proximally on the tail of the animal and allowed to fit loosely for free movement of the tail. A volume–pressure recording (VPR) cuff was placed distally behind the occlusion cuff. After that, about three to six stable measurements of blood pressure were taken, and the averages of the readings were calculated.

At the end of the experimental period (5 months) animals were anesthetized with sodium pentabarbital (100 mg/kg i.p.) and killed by exanguiation (when withdrawal reflex was absent after performing a toe pinch). After the rats were killed (at 5 months old), the abdominal aorta was removed from the animals for histological study. Aortic samples removed were fixed in 10 % formaldehyde solution for 24 hours and processed for paraffin sections of 5 micron thickness. The sections were stained with Hematoxylin and Eosin and examined under binocular light microscope (Zeiss) (Ross, M.H. *et al.*, 1989).

Statistical Analysis

Data generated from this study was analyzed with GraphPad Prism 5.0. Results are shown as the mean ± SEM. Two groups were compared using a Student's t-test, whereas multiple groups were compared by ANOVA (in all cases variances between groups were found to be equal). P< 0.05 is considered significant.

3. RESULTS

The blood pressure of 6 months old offspring (female and male) from rat dams exposed to nicotine during gestation and lactation was determined. The blood pressure of male pups was significantly higher (P<0.01) when compared with the control group (MAP= 111 ± 11.55 mm Hg vs 129 ± 12.66 mm Hg in control and the nicotine group, respectively; figure 1B), whereas blood pressure of female offspring was not significant (P>0.05) between the control and nicotine group (figure 1A). Because of this observation all subsequent experiments were performed on male offspring only.

The MAP was significantly higher in the nicotine group (121±6.4 mm Hg; P<0.01) as well as the nicotine + vitamin C group (113.4±1.7 mm Hg; P<0.05) when compared with the control group (98.1±2.4 mm Hg; figure 2A). The MAP of the group that received vitamin C after weaning (97.6±2.9 mm Hg; P>0.05) was not significantly different from that of the control group (98.1±2.4 mm Hg), but it was significantly (P<0.01) less than that of the nicotine group (121±6.4; figure 2B). This shows that vitamin C administration after weaning reduces the nicotine induced increase in MAP

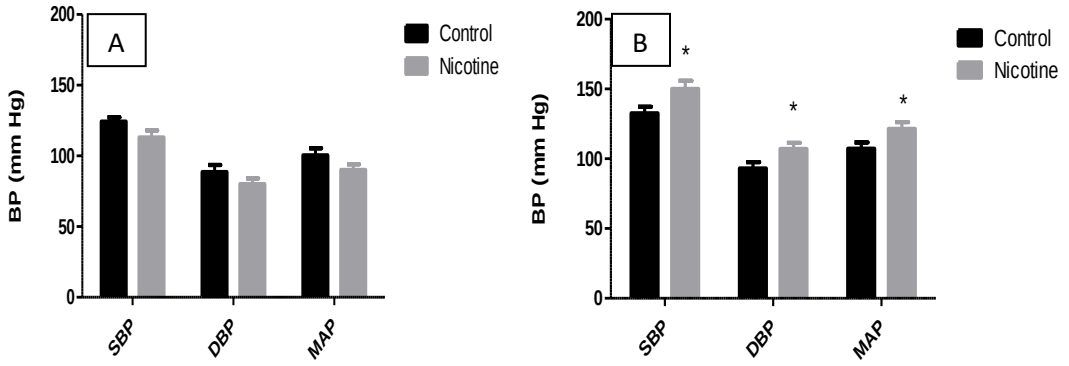


Figure 1: The effect of maternal nicotine exposure during gestation and lactation on the blood pressure of offspring at 6 months. Female (A), Male (B), SBP= Systolic blood pressure, DBP= Diastolic blood pressure, MAP= Mean arterial pressure. Data is presented as the mean ± S.E.M, *P < 0.05.

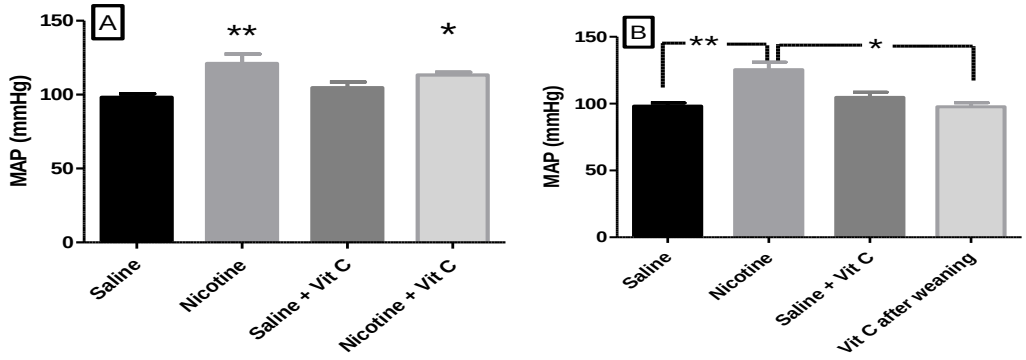


Figure 2: Mean arterial pressure in the different experimental groups at 5 months.
(A) Maternal vitamin administered to the nicotine group during pregnancy and lactation. (B) Vitamin C administration to the nicotine group after weaning. Data is presented as mean ± SEM,*P < 0.05, **P < 0.01 vs control.
(B)

Figure 3 show the histology of the control (saline) and saline + vitamin C group. Microscopic evaluation of aortic tissue in the control (saline) and saline + vitamin C group showed regularity of the aorta wall and a normal arrangement of the three layers (tunica adventitia, tunica media and tunica intima), as well as the normal linear arrangement of smooth muscle cells (SMC) and endothelial cells (EC). Meanwhile, in the nicotine, nicotine + vitamin C groups, as well as in the group that received vitamin C after weaning, aortic architecture showed irregular and abnormal alignment of the aorta wall with an irregular arrangement of the vascular smooth muscle cells (VSMCs) at the tunica media layers.

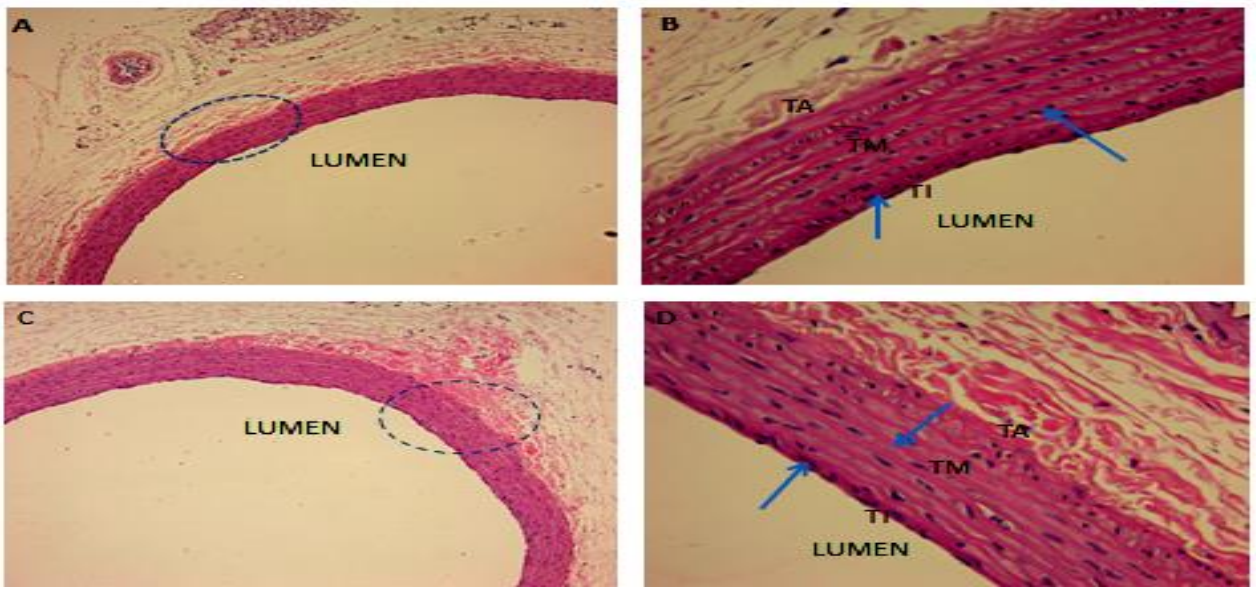
4. DISCUSSION

Previous studies have indicated that the nicotine present in cigarette smoke has led to an increase in blood pressure and heart rate during smoking (Middlekauff, H. R. *et al.,* 2014). Furthermore, it was also established that maternal exposure to nicotine induces hypertension in male offspring (Xiao, D. *et al.,* 2014).

My preliminary study demonstrated that maternal nicotine exposure during gestation and lactation increased blood pressure of male offspring but not of female offspring (figure 4.1); hence all my subsequent experiments were performed only on male offspring. These results correspond to studies conducted by Tao *et al.,* (2013) and Xiao *et al.,* (2008), where that BP was higher in the adult male offspring than in the female offspring as a result of nicotine exposure during pregnancy and lactation. This suggests a protective function of female sex hormones, such as estrogen, on perinatal exposure to nicotine induced hypertension. Xiao *et al.,* (2013) suggested that estrogen has a role in the sex difference of perinatal nicotine-induced programming of vascular dysfunction. They hypothesized that estrogen may counteract heightened reactive oxygen species production, resulting in protection of females from developmental programming of the hypertensive phenotype in adulthood.

Ciarka *et al.,* (2005) hypothesized that nicotine elevates peripheral sympathetic nerve activity to smooth muscle, causing vasoconstriction. In addition, heart rate is also increased due to sympathetic nervous system activation (Triposkiadis, F. *et al.,* 2009). Our results show that exposure to maternal nicotine did not raise HR although an increase in the blood pressure was observed, suggesting that the observed hypertension is not due to activation of the sympathetic nervous system. This will however require further investigation.

Many studies have also shown the effect of maternal antioxidant therapy on the other body organs. In the lung, a previous human trial has suggested that maternal intake of antioxidant (vitamin E) is associated with a reduced risk of respiratory disease (such as wheeze and asthma) of offspring (Devereux, G. *et al.,* 2006). It has also reported that administration of vitamin C and E during pregnancy could diminish adiposity in the offspring (Sen, S., & Simmons, R. A. 2010). Damage of the fetus brain caused by cell death via apoptosis is due to oxidative stress, and can be reduced by using antioxidants during pregnancy and lactation (Shirpoor, A *et al.,* 2009).



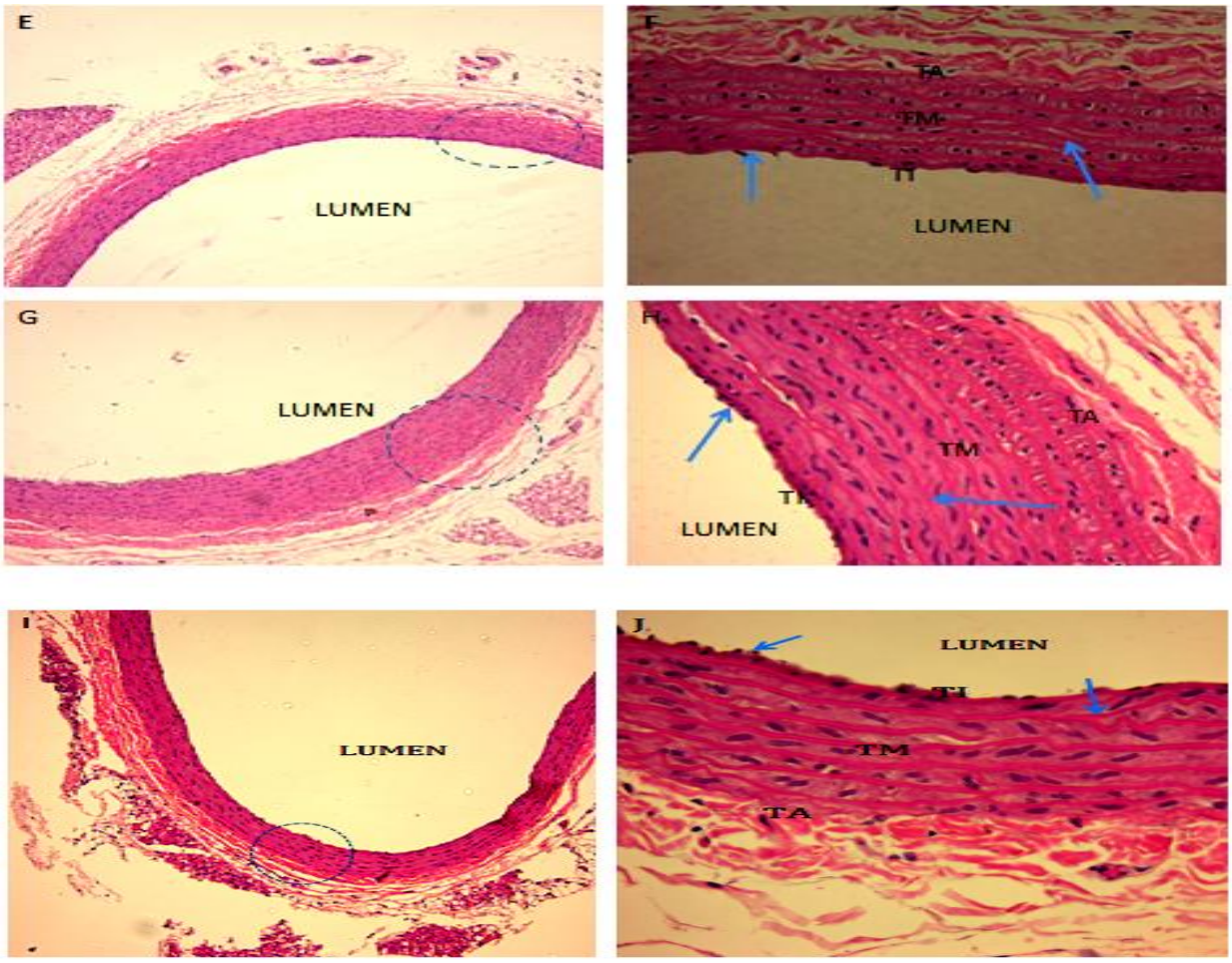


Figure 3: Transverse sections of the abdominal aorta stained with hematoxylin and eosin (H and E stain).

The results from our study show that maternal vitamin C supplementation in rats exposed to nicotine during pregnancy and lactation did not prevent development of hypertension of the male offspring. Contrary to this, vitamin C given to pups after weaning significantly improved the nicotine induced hypertension of male offspring as it leads to a reduction in the blood pressure. Our results also show that supplementation with vitamin C after weaning leads to a reduction of both systolic and diastolic blood pressure.

Our study indicates that fetus and neonatal nicotine exposure leads to increased blood pressure and morphological alterations in the layers of the aorta, characterized by irregular and abnormal alignment of the aortic wall. The alteration in the layers of the aorta tissue in the group that received the combination of nicotine and vitamin C during pregnancy and lactation, and the group that received vitamin C after weaning, was similar to the alteration of the nicotine group. This indicates that maternal vitamin C supplementation during gestation and lactation or, treating the offspring after weaning, did not prevent the effects of maternal nicotine exposure on the aorta tissue of the offspring. In contrast to our study, Maritz, G. (1993) illustrated that maternal vitamin C supplementation during pregnancy and lactation prevented the structural changes in the lung of the offspring induced by maternal nicotine exposure (Maritz, G. 1993).

It is interesting that, although the alteration in aortic structure persisted, supplementation with vitamin C did reduce nicotine induced hypertension in our study. Grossman *et al.*, (2001), suggested that vitamin C modifies the redox state of soluble guanylyl cyclase, activating cyclic GMP–dependent K-channels that hyperpolarize VSMC, inducing vasodilation. In view of the fact that the structure of the aortic wall in the nicotine + vitamin C group resembles that of the nicotine group, it will be of interest to see how vascular function is affected in the nicotine + vitamin C group when the animals become older. The vitamin C induced protection can perhaps be attributed to the fact that vitamin C is water soluble while nicotine is water as well as lipid soluble (Yildiz, D. 2004). This may reduce the capacity of vitamin C to protect the aorta against the oxidant effects of nicotine in aorta wall. Thus, a combination of vitamin C and fat soluble vitamins with anti-oxidant properties, such as vitamin E or vitamin A, is recommended.

5. CONCLUSION

Our study demonstrated that maternal nicotine exposure during gestation and lactation increased blood pressure of male offspring but not of female offspring and caused an alteration in the wall structure of the abdominal aorta, thus providing insight into the mechanisms underlying the increased prevalence hypertension in children exposed to cigarette smoke in utero. The supplementation with vitamin C after weaning leads to a reduction of the hypertension. To our knowledge this is the first time that it is shown that hypertension induced by nicotine exposure during pregnancy and lactation, can be attenuated or even reversed. It will also be of interest to determine whether epigenetic changes occurred.

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