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Adverse effects of fetal exposure of electronic-cigarettes and high-fat diet on male neonatal hearts

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Abstract

Epidemiological studies have shown an increased risk of cardiovascular diseases in children born to mothers who smoked during pregnancy. The cardiovascular risk in the offspring associated with *in utero* nicotine exposure is further exaggerated by maternal obesity. The consumption of electronic cigarettes (e-cigarettes) is alarmingly increasing among adolescents and young adults without the knowledge of their harmful health effects. There has also been a substantial increase in e-cigarette use by women of reproductive age. This study investigates the detrimental effects of gestational exposure of e-cigarette and a high-fat diet (HFD) on neonatal hearts. Time-mated pregnant mice were fed a HFD and exposed to saline or e-cigarette aerosol with 2.4% nicotine from embryonic day 4 (E4) to E20. We demonstrated that *in utero* exposure of e-cigarettes and HFD from E4 to E20 triggers cardiomyocyte (CM) apoptosis in the offspring at postnatal day1 (PND1), PND3, and PND14. Induction of CM apoptosis following gestational exposure of e-cigarettes and HFD was associated with inactivation of AMP-activated protein kinase (AMPK), increased cardiac oxidative stress coupled with perturbation of cardiac BAX/ BCL-2 ratio and activation of caspase 3 at PND 14. Electron microscopy further revealed that left ventricles of pups at PND14 after e-cigarette exposure exhibited apoptotic nuclei, convoluted nuclear membranes, myofibrillar derangement, and enlarged mitochondria occasionally showing signs of crystolysis, indicative of cardiomyopathy and cardiac dysfunction. Our results show profound adverse effects of prenatal exposure of e-cigarette plus HFD in neonatal hearts that may lead to long-term adverse cardiac consequences in the adult.

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Deceleration of competing Interest

The authors declare that they have no conflict of interest.

Keywords

E-cigarette; Nicotine; High-fat diet; Oxidative stress; Cardiomyocyte apoptosis; Mice

1. Introduction

Fetal exposure to nicotine is a major public health concerns since more than 15% of women smoke during pregnancy in the United States despite targeted public health education that pregnant women should not smoke (World Health Organization, 2013). *In utero* tobacco exposure from either maternal active tobacco use or maternal second hand tobacco exposure is associated with a variety of adverse outcomes, including obesity, neurodevelopmental disorders, premature birth, low birth weight, stillbirth, placental abruption, and sudden infant death (Farber et al., 2015; Peterson and Hecht, 2017). Epidemiological studies have clearly shown an increased risk of cardiovascular disease in children born to mothers who smoked during pregnancy (Beratis et al., 1996; Blake et al., 2000; Cohen et al., 2008). There is growing evidence that fetal nicotine exposure during pregnancy causes cardiovascular disorders later in life in several different animal models (Gao et al., 2008; Lawrence et al., 2008; Tao et al., 2013; Xiao et al., 2007b; Xiao et al., 2016; Yu et al., 2016).

The cardiovascular risk in the offspring associated with *in utero* nicotine exposure is further exaggerated by maternal obesity. There are strong associations between maternal obesity and an increase in the offspring's risk of obesity, coronary heart disease, stroke, type 2 diabetes and asthma (Chandrasekaran and Neal-Perry, 2017; Godfrey et al., 2017). Furthermore, maternal pre-pregnancy obesity and excessive weight gain during pregnancy are important risk factors for an adverse *in utero* environment and long-term detrimental cardiovascular and metabolic outcomes in the offspring (Gaillard, 2015). *In utero* exposure to a HFD can also result in genome-wide changes in DNA methylation with corresponding alterations in gene expression that persists throughout life, thus, potentially contributing to the development of metabolic disease later in life (Seki et al., 2017). Prenatal exposure to a maternal HFD can also cause changes in cardiac histone signature in offspring suggesting a diet-mediated epigenetic reprogramming of cardiac tissue *in utero* (Upadhyaya et al., 2017).

Because of the recent introduction of e-cigarettes, the development of cardiac abnormalities after *in utero* exposure of nicotine delivered *via* e-cigarettes in combination with a HFD in neonates and its long-term consequences in the adult has not been examined. There is accumulating evidence that prenatal nicotine exposure leads to severe oxidative stress (Bruin et al., 2008a; Bruin et al., 2008b; Xiao et al., 2016) that has a significant pathophysiological role in inducing cardiomyocyte (CM) apoptosis, cardiovascular remodeling and cardiac dysfunction later in life (Franco et al., 2008; Touyz and Briones, 2011). Earlier studies also consistently reveal that CM apoptosis plays a pivotal role in the morphogenesis and remodeling of mammalian heart during the first 2 postnatal weeks and serves as a potential mechanism in the development of cardiac failure of either ischemic or non-ischemic origin (Fernandez et al., 2001; Foo et al., 2005; Kajstura et al., 1995; Lee and Gustafsson, 2009; Mandl et al., 2011; Moorjani et al., 2006; Scarabelli and Gottlieb, 2004; Wencker et al., 2003).

However, to our knowledge, there are no studies looking at the effects of prenatal e-cigarette and HFD exposure on neonatal hearts. Unlike conventional cigarettes, there are no warning labels on e-cigarettes cautioning pregnant women against using them. In the current study, using our newly developed e-cigarette aerosol generation and rodent exposure system (patent number PCT/US17/54133), we tested the emerging hypothesis that exposure of e-cigarette and HFD to pregnant mice can trigger CM apoptosis and other ventricular structure abnormalities in the offspring indicative of cardiomyopathy and cardiac dysfunction.

2. Materials and methods

2.1. Mice and tissue preparation

Time-mated pregnant mice were purchased from Charles River Laboratories (Wilmington, MA) and housed singly in our animal facility under controlled temperature (22 °C) and photoperiod (12 h light, 12 h dark) with food and water *ad libitum*. These mice were fed a HFD with 60% of calories derived from fat consisting of 26.2% protein, 26.3% carbohydrate, and 34.9% fat (D12492, Research Diets, New Brunswick, NJ) and exposed to e-cigarette aerosol with 2.4% nicotine from E4 to E20. We used BluCig Plus Classic Tobacco, a popular flavor with 2.4% nicotine purchased from the BluCig website. The rational for using the combination of two common life style factors such as nicotine and HFD was based on the results of our earlier study, which demonstrated that nicotine in combination with a HFD but not with a normal chow diet (NCD) generated greater oxidative stress and triggered CM apoptosis in male mice (Sinha-Hikim et al., 2017). In other studies, we further demonstrated that combined treatment with nicotine and HFD but not with NCD caused hepatic steatosis (Friedman et al., 2012) and skeletal muscle abnormalities (Sinha-Hikim et al., 2014) in male mice.

As a control, we used a separate chamber delivering saline aerosol (AeroDeliver™ Afasci Inc., Burlingame, CA) (Shao et al., 2013). To deliver chronic intermittent e-cigarette aerosol, the device was activated for 4 s per puff, 3 puffs per vaping episode with an inter-puff interval of 30 s; one vaping episode every 30 min. Mice were exposed to intermittent e-cigarette (2.4%) or saline aerosol for 12 h (2100–0900). Mice were returned to their home cages during the light phase of 12 h (0900 to 2100) and no aerosol was delivered. Mice were exposed to e-cigarettes daily for 16 days of the study period (E4-E20). Animal handling and experimentation were in accordance with the recommendation of the American Veterinary Medical Association and were approved by the Charles R. Drew University School of Medicine and Science Institutional Animal Care and Use Committee (IACUC).

To detect the plasma nicotine levels, 3 pregnant mice were sacrificed by decapitation 30 min after the last e-cigarette with nicotine or saline exposure for 16 days on E20. Blood samples were collected form each animal into tubes containing EDTA. Plasma was sent to the UCSF Clinical Pharmacology Laboratory for measurements of nicotine by gas chromatography–mass spectrometry (GC–MS), as described previously (Jacob 3rd et al., 1991). Briefly, deuterium-labeled nicotine (nicotine-d_9) is used as the internal standard. The triple quadrupole (tandem) mass spectrometer is operated in the positive ion mode using chemical ionization with isobutane as the reagent gas. Quantitation is achieved using selected reaction monitoring. The lower limit of detection for plasma nicotine is 1 ng/mL.

Pups were euthanized on postnatal day 1 (PND1), PND3, and PND14 following *in utero* exposure of e-cigarette and HFD. Special emphasis was given to PND14, which constitutes a critical time window at which CM apoptosis plays a pivotal role in the morphogenesis and remodeling of the heart (Fernandez et al., 2001; Foo et al., 2005; Lee and Gustafsson, 2009; Mandl et al., 2011; Scarabelli and Gottlieb, 2004; Wencker et al., 2003). Given the gender specific effects of *in utero* nicotine exposure with male pups being more susceptible to apoptosis than female pups (Xiao et al., 2007a; Xiao et al., 2016) we elected to use only male pups.

For the analysis of the cardiac outcomes, we used ten randomly selected male pups from 5 different litters in each group. Ten offspring (2 pups/litter) were euthanized at each time point and 5 litters were represented at each of time point. Groups of 15 pregnant dams were treated with saline or e-cigarette. At autopsy, ventricles from each pup in each group were quickly removed and fixed with 4% formalin for terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate nick end labeling (TUNEL) and immunohistochemical studies. We also performed transmission electron microscopy (TEM) for left ventricles at PND14. Left ventricles from pups at PND14 were carefully dissected out and fixed in 2.5% glutaraldehyde, post-fixed in 1% osmium tetroxide, and embedded in Epon 812 as described previously (Friedman et al., 2012; Sinha-Hikim et al., 2017). Selected tissue blocks were sectioned with an LKB ultramicrotome to obtain thin sections, which were then stained with uranyl acetate and lead citrate, and examined with a Hitachi electron microscope (Hitachi, Indianapolis, IN, USA).

2.2. CM apoptosis

Visualization of apoptotic CMs was achieved in formalin-fixed, paraffin-embedded ventricular sections by the TUNEL technique (Sinha-Hikim et al., 2017; Sinha-Hikim et al., 2011a) using an ApopTag-peroxidase kit (Chemicon International, Inc., San Francisco, CA). Slides were counterstained with methyl green for detection of non-apoptotic nuclei. We also performed TEM to confirm the apoptotic nature of the cell death and the identity of the dying cardiomyocytes (Sinha-Hikim et al., 2017; Sinha-Hikim et al., 2011a). Quantitation of CM nuclei (both apoptotic and non-apoptotic) was carried out using an unbiased 2-dimension rule (Cruz-Orive and Weibel, 1990) as described previously (Sinha-Hikim et al., 2017; Sinha-Hikim et al., 2011a). For each ventricle at least 10 grid fields were examined. The rate of CM apoptosis was expressed as the percentage of the TUNEL-positive apoptotic nuclei per total nuclei (apoptotic plus non apoptotic) present within the reference area (Sinha-Hikim et al., 2017; Sinha-Hikim et al., 2011a).

2.3. Immunohistochemical analysis

Immunohistochemical analyses were performed in formalin fixed, paraffin-embedded ventricular sections as described previously (Sinha-Hikim et al., 2017; Sinha-Hikim et al., 2011a) using mouse monoclonal 4-hydroxynonenal protein adducts (4-HNE; 1:100; Oxis International Inc., Foster City, CA) and rabbit polyclonal phospho-AMP-activated protein kinase (p-AMPK; 1:100), BAX (1:50), BCL-2 (1:50) from Santa Cruz Biotechnology, Santa Cruz, CA), and active caspase 3 (1:50; Cell Signaling Technology, Beverly, MA) primary antibodies. Immunoreactivity was detected using biotinylated anti-mouse or anti-rabbit

IgG secondary antibody followed by avidin-biotinylated horseradish peroxidase complex, and visualized with diaminobenzidine tetrahydro-chloride (DAB) per the manufacturer's instructions (VECTASTAIN Elite ABC Rabbit IgG kit, Burlingame, CA). Slides were counterstained with hematoxylin. For negative controls, sections were treated with mouse or rabbit IgG, and no signals were detected. Staining intensity was quantified by computerized densitometry using the ImagePro Plus, version 5.1 software (Media Cybernetics, Silver Spring, MD) as described previously (Sinha-Hikim et al., 2017).

2.4. Statistical analyses

Statistical analyses were performed using the SigmaStat 2.0 Program (Jandel Corporation, San Rafael, CA, USA). Data were presented as mean ± SEM. We used one way analysis of variance (ANOVA) followed by a Tukey's test to assess the statistically significant differences among various treatment groups. Our statistical analyses were based on 5 litter per experimental group at each time point. Differences were considered significant if $p < 0.05$.

3. Results

3.1. Plasma nicotine levels

The average plasma concentrations of nicotine at the end of treatment was 48.3 ± 5.4 (ng/mL), which is similar to the clinically relevant concentrations found in habitual cigarette smokers or users of nicotine containing chewing gums (Wu et al., 2015). In contrast, the concentrations of plasma nicotine in pregnant dams that received saline were undetectable.

3.2. In utero exposure of e-cigarette plus HFD triggers fetal CM apoptosis and causes ventricular ultrastructural abnormalities in neonatal pups

We next analyzed the initiation of apoptosis in ventricles at PND1, PND3, and PND14 following *in utero* exposure of e-cigarette and HFD. Minimal CM apoptosis was detected in control pups (Fig. 1). In contrast, gestational exposure of e-cigarette and HFD resulted in a striking increase in the incidence of CM apoptosis at PND3 and PND14 (Fig. 1A). We also quantitated the incidence of fetal CM apoptosis, expressed as the percentage of TUNEL-positive nuclei per total nuclei (apoptotic plus non-apoptotic nuclei). The rate of CM apoptosis was essentially similar in controls for PND1 (0.89 ± 0.31), PND3 (0.86 ± 0.24), and PND14 (1.67 ± 0.14). In contrast, gestational exposure of e-cigarettes and HFD led to a striking increase in the incidence of CM apoptosis at PND3 (28.8 ± 1.5-fold increase, $P < 0.05$) (Fig. 1C). and PND14 (21.1 ± 1.3-fold increase, $P < 0.05$) (Fig. 1D). when compared with respective controls.

We next performed TEM to evaluate left ventricular myofibrillar architecture between saline-and e-cigarette-exposed groups at PND14 (Fig. 2). CMs from pups born to mothers exposed to saline and HFD exhibited normal myofibrillar architecture and sarcomeric pattern with normal nuclei and abundant mitochondria. In contrast, CMs of pups after gestational exposure of e-cigarettes and HFD exhibited both nuclear as well as cytoplasmic abnormalities. The most predominant nuclear abnormalities included shrunken nuclei with chromatin condensation and fragmentation (characteristic of apoptosis) and nuclear

malformation with extensively convoluted nuclear membrane. The most striking cytoplasmic abnormalities were myofibrillar thinning and derangement and enlarged mitochondria occasionally showing signs of crystolysis.

3.3. Gestational exposure of e-cigarettes plus HFD inactivates AMPK and triggers oxidative stress in neonatal hearts

Given that AMPK plays a pivotal role regulating CM apoptosis and in the adaptive response to CM stress (Guo et al., 2015; Qi and Young, 2015; Zhang et al., 2009; Zhuo et al., 2013), we next examined, whether induction of fetal CM apoptosis is associated with inactivation of AMPK at PND14 following *in utero* exposure of e-cigarettes and HFD. Compared with controls, *in utero* exposure of e-cigarettes and HFD led to a decrease in phospho-AMPK immunoreactivity (Fig. 3A).

We further examined the *in vivo* expression profiles of a 4-HNE, a lipid peroxidation product that is a biomarker of oxidative stress (Kohen and Nyska, 2002; Tam et al., 2003) in the ventricles of pups at PND14. Compared with mice born to dams exposed to HFD and saline aerosol, where a modest expression of 4-HNE immunoreactivity is detected, we found a striking increase in 4-HNE immunoreactivity at PND14 (Fig. 3B) after *in utero* exposure of e-cigarette aerosol and HFD. These findings were further substantiated by densitometric analysis (Fig. 3. C and D).

3.4. Gestational exposure of e-cigarettes and HFD perturbs BAX/BCL-2 ratio and triggers caspase 3 activation

Given that the ratio of anti-apoptotic and pro-apoptotic BCL-2 family members such as BAX/BCL-2 constitutes a rheostat that sets the thresholds for susceptibility of apoptosis in the intrinsic pathway signaling (Danial and Korsmeyer, 2004), which is also the key pathway of fetal CM apoptosis (Sinha-Hikim et al., 2011b), we examined the expression profiles of BAX, BCL-2, and active caspase 3. Since *in utero* exposure of saline and HFD neither affect incidence of CM apoptosis nor ventricular BAX, BCL-2, and active caspase 3 immunoreactivity is seen at various postnatal days, we only used ventricular sections at PND3 as controls. Compared to control ventricles, we found a significant increase in ventricular BAX and a decrease in BCL-2 immunoreactivity at PND14 (Fig. 4A upper and middle panels, B, C). Perturbation of the ventricular BAX/BCL-2 ratio at PND14 following *in utero* exposure of e-cigarette was further associated with activation of caspase 3, as evidenced by an increase in the immunostaining of active caspase 3 (Fig. 4A bottom panel, D).

4. Discussion

There is growing evidence from animal experimentation that fetal nicotine exposure during pregnancy can cause a variety of adverse outcomes in the developing hearts resulting in cardiovascular disorders later in life (Lawrence et al., 2011; Lawrence et al., 2008; Tao et al., 2013; Xiao et al., 2015; Xiao et al., 2016). In addition, *in utero* exposure to a HFD can also result in genome-wide changes in DNA methylation with corresponding alterations in gene expression that persists throughout life, thus, potentially contributing to the development

of metabolic disease later in life (Seki et al., 2017). Prenatal exposure to a maternal HFD further initiates changes in cardiac histone signature in offspring suggesting a diet-mediated epigenetic reprogramming of cardiac tissue *in utero* (Upadhyaya et al., 2017). In a recent study, it has been reported that prenatal exposure to e-cigarettes led to a reduction of significant body weight gain in female offspring and a slight decrease in fertility in male offspring (Wetendorf et al., 2019). However, to our knowledge, there are no studies looking at the effects of prenatal e-cigarette and HFD exposure on neonatal hearts. In this study, using our newly developed e-cigarette aerosol generation and rodent exposure system, we studied the detrimental effects of nicotine delivered through e-cigarettes and HFD on the developing hearts. Our data demonstrate that 1) *in utero* exposure of nicotine in e-cigarettes plus HFD triggers fetal CM apoptosis and causes ventricular ultrastructural abnormalities indicative of cardiomyopathy in neonatal hearts and 2) the detrimental effects of gestational exposure of e-cigarette and HFD on CM apoptosis are associated with inactivation AMPK, increased cardiac oxidative stress coupled with perturbation of cardiac BAX/BCL-2 ratio and activation of caspase 3.

Consistent with a pivotal role of AMPK in myocardial cell apoptosis, here we show that gestational exposure of e-cigarettes and HFD caused significant inhibition (dephosphorylation) of ventricular AMPK. This is in accord with our earlier reports that combined with a HFD, nicotine causes inhibition of AMPK in the liver (Friedman et al., 2012), in the skeletal muscle (Sinha-Hikim et al., 2014) and in cardiac ventricles (Sinha-Hikim et al., 2017) of adult mice. Of note, inhibition of AMPK can also generate oxidative stress in a variety of cell systems, including cardiac myocytes (Jeon et al., 2012; Jia et al., 2011; Park et al., 2012). However, we cannot rule out the possibility that other factors such as downregulation of its own could have also contributed to decreased phospho-AMPK caused by gestational exposure of e-cigarette and HFD. Thus, it is possible that inhibition of AMPK, through generation of oxidative stress, could trigger CM apoptosis in pups after prenatal exposure of e-cigarettes and HFD.

Oxidative stress plays a major role in initiation of apoptosis in various cell types, including cardiomyocytes (Guo et al., 2015;Sinha-Hikim et al., 2011a; Sun et al., 2006; Zhou et al., 2010). This study showed that compared to controls, gestational exposure of e-cigarettes and HFD generated greater oxidative stress, as evidenced by increase ventricular 4-HNE expression, which agrees with our previous data indicating that combined with a HFD, nicotine is capable of generating oxidative stress in the adult heart (Sinha-Hikim et al., 2017). Thus, it is likely that increased oxidative stress following *in utero* exposure of e-cigarettes and HFD could contribute to increased neonatal CM apoptosis. This is also consistent with our report indicating that gestational exposure of cocaine through generation of oxidative stress caused CM apoptosis in rat pups, which can be prevented by suppression of oxidative stress (Sinha-Hikim et al., 2011a).

The BCL-2 family of proteins governs the mitochondria-dependent intrinsic apoptotic pathway (Danial and Korsmeyer, 2004). Our ventricular immunohistochemical data clearly show an increase in BAX and a decrease in BCL-2 immunoreactivity at PND14 following gestational exposure of e-cigarettes and HFD. Perturbation of the ventricular BAX/ BCL-2 ratio following *in utero* exposure of e-cigarettes was further associated with activation of

caspase 3, as evidenced by an increase in the immunostaining of active caspase 3. Thus, it is likely that the mitochondria-dependent intrinsic apoptotic pathway plays an important role in fetal CM apoptosis triggered by *in utero* exposure of e-cigarettes and HFD. This corroborates our previous works indicating that mitochondrial-dependent intrinsic pathway signaling constitutes a critical component of apoptotic signaling in fetal CMs trigged by *in utero* cocaine exposure (Sinha-Hikim et al., 2011a) as well as in nicotine plus HFD-induced CM apoptosis in adult mice (Sinha-Hikim et al., 2017).

One potential limitation of our study is that we did not have an additional control group exposed to e-cigarette aerosol with no nicotine (0%) to rule out the possibilities that the cardiac changes in the e-cigarette with nicotine are indeed attributed to nicotine. In this context, it is worth noting here, that in a recent study, we found that Apolipoprotein-E knockout (ApoE−/−) mice exposed to only e-cigarette with nicotine (2.4%) not without nicotine (0%) for 12 weeks had pronounced adverse effects on the heart (Espinoza-Derout et al., 2019). We also found in that study that these mice exposed to e-cigarette in the absence of nicotine (0%) exhibited no cardiac injury and was comparable to mice that were exposed to saline aerosol. In a separate study, we also found that 12 weeks exposure of e-cigarette with no nicotine (0%) had no discernable effect on the liver of these mice (Hasan et al., 2019). Thus, the detrimental effects of fetal exposure of e-cigarette on the neonatal hearts are likely due to the presence of nicotine. However, this clearly merits further investigation.

In summary, our findings contradict the popular belief that e-cigarettes are safe and highlight multiple detrimental effects of gestational exposure of e-cigarettes and HFD on the fetal hearts. We conclude that 1) *in utero* exposure of nicotine in e-cigarettes plus HFD triggers fetal CM apoptosis and causes ventricular ultrastructural abnormalities indicative of cardiomyopathy in neonatal hearts and 2) the detrimental effects of gestational exposure of e-cigarette and HFD on CM apoptosis are associated with an inactivation of AMPK, increased cardiac oxidative stress coupled with perturbation of cardiac BAX/BCL-2 ratio and activation of caspase 3. We surmise that prenatal exposure of e-cigarette plus HFD is likely to be a very toxic combination in infants that could even led to long-term adverse cardiac consequences in the adult.

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References

Beratis NG, et al. , 1996. Increased blood pressure in neonates and infants whose mothers smoked during pregnancy. J. Pediatr. 128, 806–812. [PubMed: 8648540]

Blake KV, et al. , 2000. Maternal cigarette smoking during pregnancy, low birth weightand subsequent blood pressure in early childhood. Early Hum. Dev. 57, 137–147. [PubMed: 10735460]

Bruin JE, et al. , 2008a. Maternal nicotine exposure increases oxidative stress in the offspring. Free Radic. Biol. Med. 44, 1919–1925. [PubMed: 18343235]

Bruin JE, et al. , 2008b. Fetal and neonatal nicotine exposure in Wistar rats causes progressive pancreatic mitochondrial damage and beta cell dysfunction. PLoS One 3,e3371. [PubMed: 18852877]

Chandrasekaran S, Neal-Perry G, 2017. Long-term consequences of obesity on femalefertility and the health of the offspring. Curr. Opin. Obstet. Gynecol. 29, 180–187. [PubMed: 28448277]

Cohen G, et al. , 2008. Cardiovascular stress hyperreactivity in babies of smokers and inbabies born preterm. Circulation 118, 1848–1853. [PubMed: 18852367]

Cruz-Orive LM, Weibel ER, 1990. Recent stereological methods for cell biology: abrief survey. Am. J. Phys. 258, L148–L156.

Danial NN, Korsmeyer SJ, 2004. Cell death: critical control points. Cell 116, 205–219. [PubMed: 14744432]

Espinoza-Derout J, et al. , 2019. Chronic intermittent electronic cigarette exposure induces cardiac dysfunction and atherosclerosis in apolipoprotein E (ApoE) knockoutmice. Am. J. Physiol. Heart Circ. Physiol. 317, H445–H559. [PubMed: 31172811]

Farber HJ, et al. , 2015. Protecting children from tobacco, nicotine, and tobacco smoke. Pediatrics 136, e1439–e1467. [PubMed: 26504135]

Fernandez E, et al. , 2001. Apoptosis and proliferation in the neonatal murine heart. Dev. Dyn. 221, 302–310. [PubMed: 11458390]

Foo RS, et al. , 2005. Death begets failure in the heart. J. Clin. Invest. 115, 565–571. [PubMed: 15765138]

Franco R, et al. , 2008. Glutathione depletion and disruption of intracellular ionichomeostasis regulate lymphoid cell apoptosis. J. Biol. Chem. 283, 36071–36087. [PubMed: 18940791]

Friedman TC, et al. , 2012. Additive effects of nicotine and high-fat diet on hepaticsteatosis in male mice. Endocrinology 153, 5809–5820. [PubMed: 23093702]

Gaillard R, 2015. Maternal obesity during pregnancy and cardiovascular development and disease in the offspring. Eur. J. Epidemiol. 30, 1141–1152. [PubMed: 26377700]

Gao YJ, et al. , 2008. Effects of fetal and neonatal exposure to nicotine on blood pressure and perivascular adipose tissue function in adult life. Eur. J. Pharmacol. 590, 264–268. [PubMed: 18647709]

Godfrey KM, et al. , 2017. Influence of maternal obesity on the long-term health of offspring. Lancet Diabetes Endocrinol. 5, 53–64. [PubMed: 27743978]

Guo S, et al. , 2015. Resveratrol attenuates high glucose-induced oxidative stress andcardiomyocyte apoptosis through AMPK. Mol. Cell. Endocrinol. 412, 85–94. [PubMed: 26054749]

Hasan KM, et al. , 2019. E-cigarettes and Western diet: important metabolic risk factorsfor hepatic diseases. Hepatology. 69 (6), 2442–2454. [PubMed: 30664268]

Jacob P 3rd, et al. , 1991. Selected ion monitoring method for determination of nicotine,cotinine and deuterium-labeled analogs: absence of an isotope effect in the clearanceof (S)-nicotine-3′,3′-d2 in humans. Biol. Mass Spectrom. 20, 247–252. [PubMed: 1883864]

Jeon SM, et al. , 2012. AMPK regulates NADPH homeostasis to promote tumour cellsurvival during energy stress. Nature. 485, 661–665. [PubMed: 22660331]

Jia F, et al. , 2011. AMP-activated protein kinase inhibits homocysteine-induced dysfunction and apoptosis in endothelial progenitor cells. Cardiovasc. Drugs Ther 25, 21–29. [PubMed: 21258964]

Kajstura J, et al. , 1995. Programmed cell death and expression of the protooncogene bcl-2 in myocytes during postnatal maturation of the heart. Exp. Cell Res. 219,110–121. [PubMed: 7628527]

Kohen R, Nyska A, 2002. Oxidation of biological systems: oxidative stress phenomena, antioxidants, redox reactions, and methods for their quantification. Toxicol. Pathol. 30, 620–650. [PubMed: 12512863]

Lawrence J, et al. , 2008. Prenatal nicotine exposure increases heart susceptibility to ischemia/ reperfusion injury in adult offspring. J. Pharmacol. Exp. Ther. 324, 331–341. [PubMed: 17947495]

Lawrence J, et al. , 2011. Foetal nicotine exposure causes PKCepsilon gene repression by promoter methylation in rat hearts. Cardiovasc. Res. 89, 89–97. [PubMed: 20733009]

Lee Y, Gustafsson AB, 2009. Role of apoptosis in cardiovascular disease. Apoptosis. 14, 536–548. [PubMed: 19142731]

Mandl A, et al. , 2011. Puma deletion delays cardiac dysfunction in murine heart failure models through attenuation of apoptosis. Circulation. 124, 31–39. [PubMed: 21670227]

Moorjani N, et al. , 2006. Activation of apoptotic caspase cascade during the transition to pressure overload-induced heart failure. J. Am. Coll. Cardiol. 48, 1451–1458. [PubMed: 17010810]

Park CS, et al. , 2012. Metformin reduces airway inflammation and remodeling via activation of AMP-activated protein kinase. Biochem. Pharmacol. 84, 1660–1670. [PubMed: 23041647]

Peterson LA, Hecht SS, 2017. Tobacco, e-cigarettes, and child health. Curr. Opin. Pediatr. 29, 225–230. [PubMed: 28059903]

Qi D, Young LH, 2015. AMPK: energy sensor and survival mechanism in the ischemic heart. Trends Endocrinol. Metab. 26, 422–429. [PubMed: 26160707]

Scarabelli TM, Gottlieb RA, 2004. Functional and clinical repercussions of myocyteapoptosis in the multifaceted damage by ischemia/reperfusion injury: old and new concepts after 10 years of contributions. Cell Death Differ. 11 (Suppl. 2), S144–S152. [PubMed: 15608693]

Seki Y, et al. , 2017. In utero exposure to a high-fat diet programs hepatic Hypermethylation and gene dysregulation and development of metabolic syndrome in male mice. Endocrinology. 158, 2860–2872. [PubMed: 28911167]

Shao XM, et al. , 2013. Nicotine delivery to rats via lung alveolar region-targetedaerosol technology produces blood pharmacokinetics resembling human smoking. Nicotine Tob. Res. 15, 1248–1258. [PubMed: 23239844]

Sinha-Hikim I, et al. , 2011a. Minocycline suppresses oxidative stress and attenuates fetal cardiac myocyte apoptosis triggered by in utero cocaine exposure. Apoptosis. 16, 563–573. [PubMed: 21424555]

Sinha-Hikim I, et al. , 2011b. A novel cystine based antioxidant attenuates oxidativestress and hepatic steatosis in diet-induced obese mice. Exp. Mol. Pathol. 91, 419–428. [PubMed: 21570964]

Sinha-Hikim I, et al. , 2014. Nicotine in combination with a high-fat diet causes intramyocellular mitochondrial abnormalities in male mice. Endocrinology. 155, 865–872. [PubMed: 24424058]

Sinha-Hikim I, et al. , 2017. Nicotine plus a high-fat diet triggers cardiomyocyte apoptosis. Cell Tissue Res. 368, 159–170. [PubMed: 27917437]

Sun Y, et al. , 2006. Oxidative stress in aldosteronism. Cardiovasc. Res. 71, 300–309. [PubMed: 16631148]

Tam NN, et al. , 2003. Androgenic regulation of oxidative stress in the rat prostate:involvement of NAD(P)H oxidases and antioxidant defense machinery duringprostatic involution and regrowth. Am. J. Pathol. 163, 2513–2522. [PubMed: 14633623]

Tao H, et al. , 2013. Angiotensin II-mediated vascular changes in aged offspring rats exposed to perinatal nicotine. Peptides. 44, 111–119. [PubMed: 23500520]

Touyz RM, Briones AM, 2011. Reactive oxygen species and vascular biology:implications in human hypertension. Hypertens. Res. 34, 5–14. [PubMed: 20981034]

Upadhyaya B, et al. , 2017. Prenatal exposure to a maternal high-fat diet affects histonemodification of Cardiometabolic genes in newborn rats. Nutrients. 9.

Wencker D, et al. , 2003. A mechanistic role for cardiac myocyte apoptosis in heartfailure. J. Clin. Invest. 111, 1497–1504. [PubMed: 12750399]

Wetendorf M, et al. , 2019. E-cigarette exposure delays implantation and causes reducedweight gain in female offspring exposed in utero. J. Endocr. Soc. 3, 1907–1916. [PubMed: 31598571]

World Health Organization, 2013. WHO Recommendations for the Prevention andManagement of Tobacco Use and Second-hand Smoke Exposure in Pregnancy.

Wu Y, et al. , 2015. Activation of AMPKalpha2 in adipocytes is essential for nicotine-induced insulin resistance *in vivo*. Nat. Med. 21, 373–382. [PubMed: 25799226]

Xiao D, et al. , 2007a. Fetal and neonatal nicotine exposure differentially regulatesvascular contractility in adult male and female offspring. J. Pharmacol. Exp. Ther. 320, 654–661. [PubMed: 17068201]

Xiao D, et al. , 2007b. Direct effects of nicotine on contractility of the uterine artery inpregnancy. J. Pharmacol. Exp. Ther. 322, 180–185. [PubMed: 17403992]

Xiao D, et al. , 2015. Antenatal antioxidant prevents nicotine-mediated hypertensive response in rat adult offspring. Biol. Reprod. 93, 66. [PubMed: 26224008]

Xiao D, et al. , 2016. Protective effect of antenatal antioxidant on nicotine-induced heartischemia-sensitive phenotype in rat offspring. PLoS One 11, e0150557.

Yu F, et al. , 2016. Prenatal nicotine exposure results in the myocardial fibrosis in the adult male offspring rats. Exp. Toxicol. Pathol. 68, 445–450. [PubMed: 27436000]

Zhang BB, et al. , 2009. AMPK: an emerging drug target for diabetes and the metabolicsyndrome. Cell Metab. 9, 407–416. [PubMed: 19416711]

Zhou X, et al. , 2010. Nicotine promotes cardiomyocyte apoptosis via oxidative stress and altered apoptosis-related gene expression. Cardiology. 115, 243–250. [PubMed: 20339300]

Zhuo XZ, et al. , 2013. Isoproterenol instigates cardiomyocyte apoptosis and heartfailure via AMPK inactivation-mediated endoplasmic reticulum stress. Apoptosis. 18,800–810. [PubMed: 23620435]

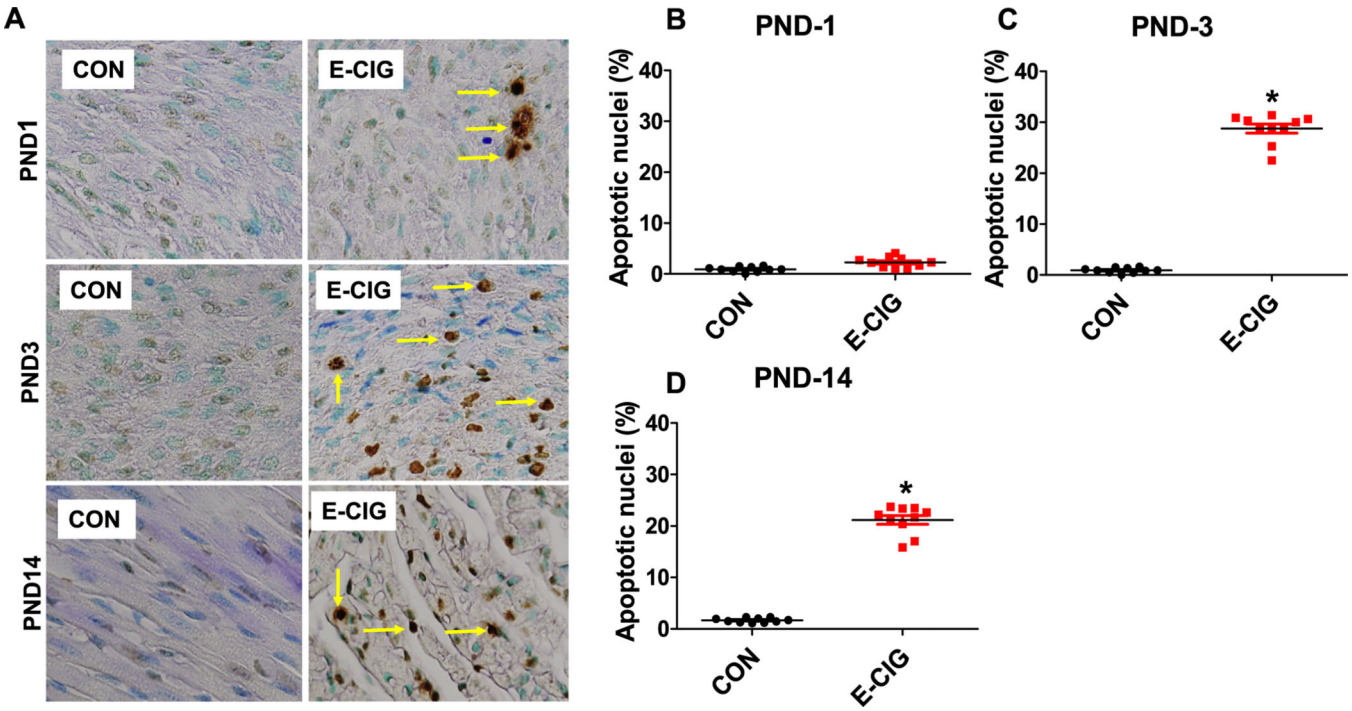

Fig. 1.

In situ detection of ventricular CM apoptosis detected by TUNEL. Incidence of CM apoptosis is minimal in controls at PND1, PND3, and PND14. In contrast, gestational exposure of e-cigarettes and HFD triggers a marked increase in the incidence of neonatal CM apoptosis at PND3 and PND14(A). For quantitation of CM apoptosis, apoptotic rate was expressed as the percentage of TUNEL-positive nuclei per total nuclei (apoptotic plus non-apoptotic nuclei) counted in a unit reference area (B,C,D). Values are means ± SEM (n = 10). * Significantly different (P <0.05) from respective controls. (Bar 25 μm).

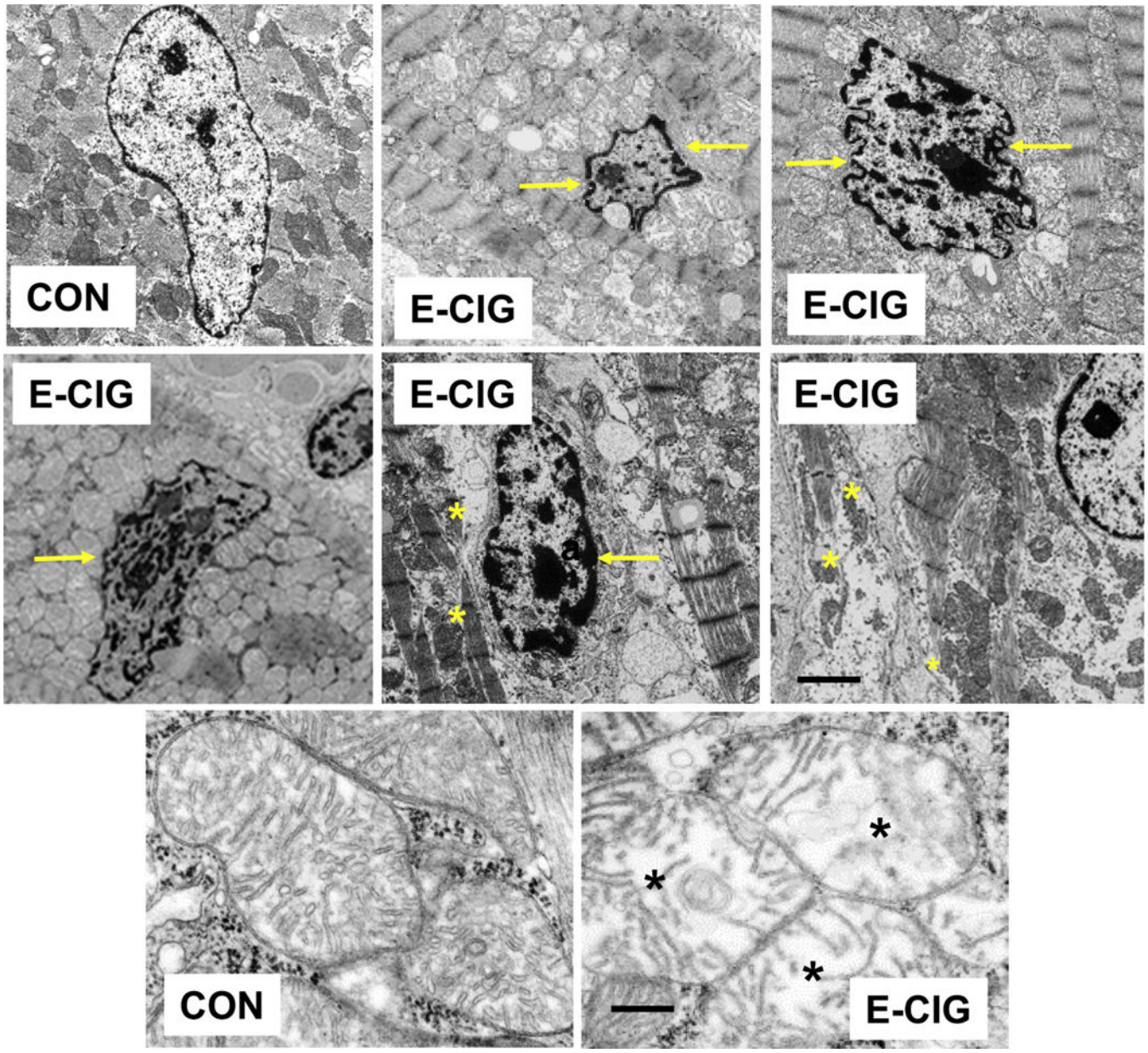

Fig. 2.

Control CMs from pups born to mothers exposed to saline and HFD exhibited normal myofibrillar architecture and sarcomeric pattern with normal nuclei and abundant mitochondria. In contrast representative TEM images of ventricles at PND14 following *in utero* exposure of e-cigarettes and HFD show varying degrees of nuclear and myofibrillar abnormalities in CMs. The most predominant nuclear abnormalities (yellow arrow) included shrunken nuclei with chromatin condensation and fragmentation (characteristic of apoptosis) and nuclear malformation with extensively convoluted nuclear membrane. The most pronounced cytoplasmic abnormalities (yellow asterisk) were myofibrillar thinning and derangement. Representative higher magnified TEM images of ventricles from pups at PND14 following *in utero* exposure of e-cigarettes and HFD show disappearance of cristae

(black asterisks) crystolysis when compared with controls (bottom panel). Bar 2 μm (upper and middle panels). Bar 0.5 μm (bottom panel).

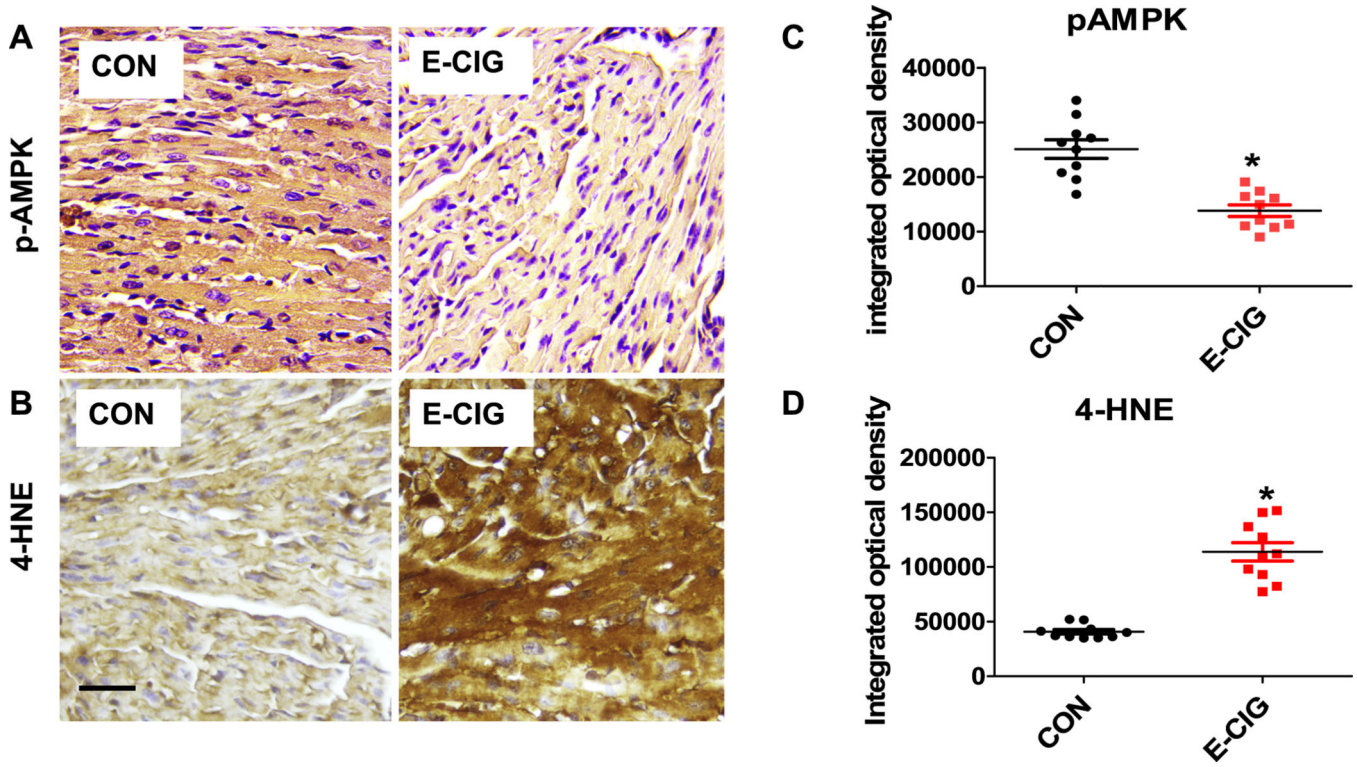

Fig. 3.

Visualization of AMPK inactivation (dephosphorylation) by immunohistochemistry. Compared with controls, *in utero* exposure of e-cigarettes and HFD results in a decrease of phospho-ventricular AMPK immunoreactivity in PND14 pups (A). *In utero* exposure of e-cigarette and HFD causes a greater oxidative stress, as evidenced by elevated ventricular 4-HNE levels, relative to pups born to mothers exposed to saline and HFD (B). Bar 25 μm. Quantitation of ventricular staining intensities of p-AMPK (C) and 4-HNE (D). Values are means ± SEM (n = 10). *Significantly different ($P < 0.05$) from respective controls.

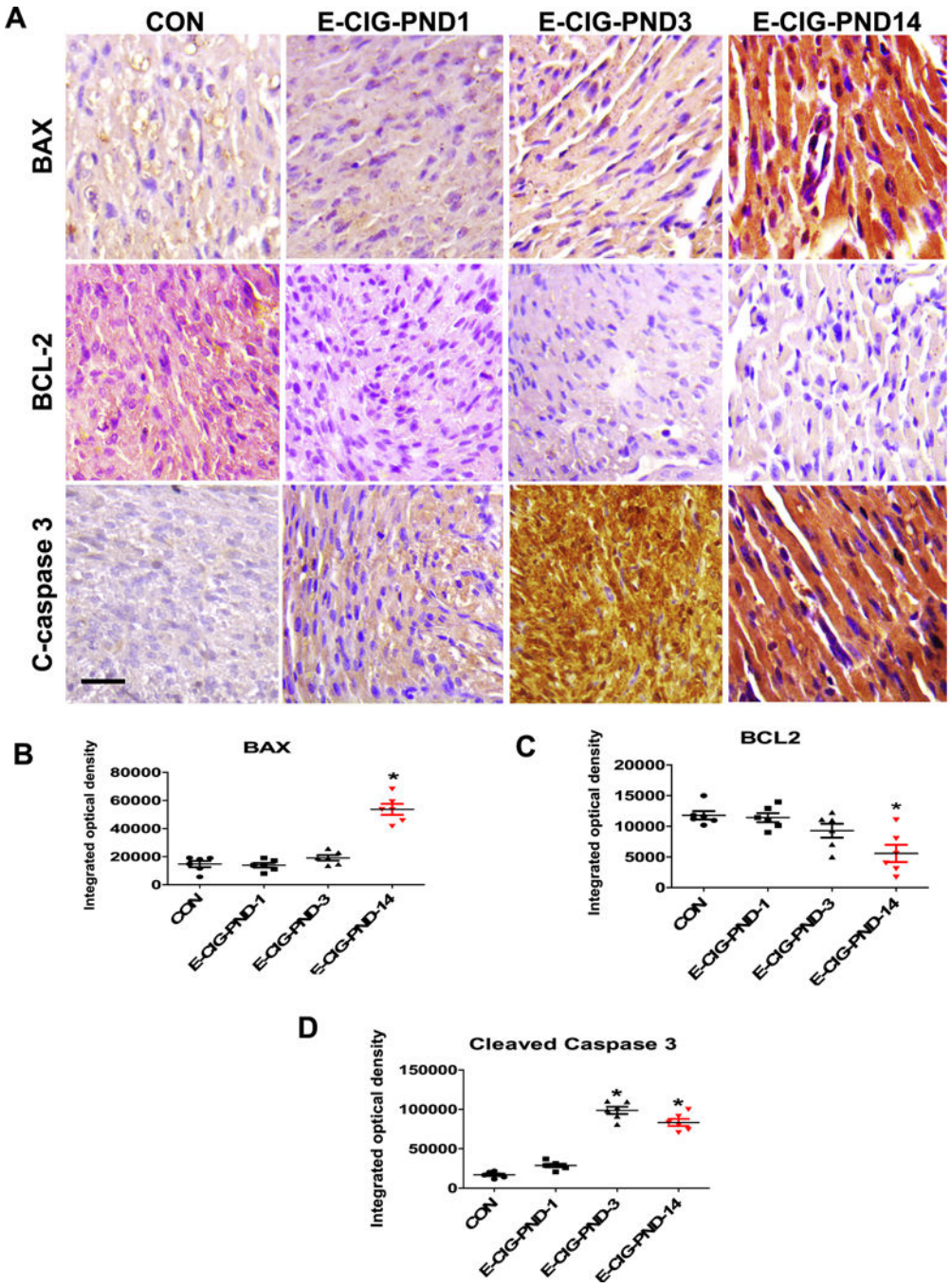

Fig. 4.

Temporal changes in the expression of ventricular BAX, BCL-2, and active Caspase 3 detected by immunohistochemistry in pups at different postnatal days following *in utero* exposure of e-cigarettes and HFD (A). Note a significant increase in BAX expression (upper panel) and a decrease in BCL-2 expression (middle panel) after gestational exposure of e-cigarettes and HFD at PND 14. Perturbation of the ventricular BAX/BCL-2 ratio at PND14 following *in utero* exposure of e-cigarettes and HFD was further associated with activation of caspase 3 in ventricles, as evidenced by an increase in the immunostaining

of active caspase 3 (bottom panel). Quantitation of ventricular staining intensities of BAX (B), BCL-2 (C), and active caspase 3 (D). Values are means ± SEM ($n = 6$). *Significantly different ($P < 0.05$) from respective controls. Bar 25 μm.