 doi:10.20944/preprints202205.0141.v1

Review

Alterations of Mitochondrial Network by Cigarette and e-Cigarette Vaping

Manasa Kanithi 1, Sunil Junapudi 2, Syed Islamuddin Shah 3, Matta Reddy Alavala 4, Ghanim Ullah *3 and Bojjibabu Chidipi *5

1 College of Osteopathic Medicine, Michigan State University, East Lansing, MI -48824, USA (email: kmk12345@gmail.com)
2 Department of Pharmaceutical Chemistry, Geethanjali College of Pharmacy, Cherryal, Keesara, Medchalmalkajgiri District, Telangana -501301, India (email: suniljunapudi@gmail.com)
3 Department of Physics, University of South Florida, Tampa, FL -33620, USA (email: islamuddinn@yahoo.com, gullah@usf.edu)
4 Adikavi University Road, Rajahmundry, Andhra Pradesh 533296, India (email: alavalareddy1@gmail.com)
5 Morsani College of Medicine, University of South Florida, Tampa, FL-33612, USA (email: chidipib@usf.edu)
* Correspondence: chidipib@usf.edu (B.C.); gullah@usf.edu (G.U.)

Abstract: Toxins present in cigarette and e-cigarette smoke constitute a significant cause of illnesses and are known to have fatal health impacts. Specific mechanisms by which toxins present in smoke impair cell repair are still being researched and are of prime interest for developing more effective treatments. Current literature suggests toxins present in cigarette smoke and aerosolized e-vapor trigger abnormal intercellular responses, damage mitochondrial function, and consequently disrupt the homeostasis of the organelle's biochemical processes by increasing reactive oxidative species. Increased oxidative stress sets off a cascade of molecular events, disrupting optimal mitochondrial morphology and homeostasis. Furthermore, smoking-induced oxidative stress may also amalgamate with other health factors to contribute to various pathophysiological processes. An increasing number of studies show that toxins may affect mitochondria even though exposure to secondhand or thirdhand smoke. This review assesses the impact of toxins present in tobacco smoke and e-vapor on mitochondrial health, networking, and critical structural processes including mitochondria fission, fusion, hyperfusion, fragmentation, and mitophagy. The efforts are focused on discussing current evidence linking toxins present in first, second, and thirdhand smoke to mitochondrial dysfunction

Keywords: Cigarette smoking; e-cigarette smoking; mitochondria; fusion; fission

1. Introduction.

Cigarette smoking (CS), E-cigarette (EC) vaping, and other types of exposure to environmental tobacco smoke, including second and thirdhand smoke, are dangerous to human health, causing diseases that affect every organ system[1-4]. Despite thorough documentation of the extensive damages caused by smoking, it continues to be one of the most prevalent public health concerns worldwide, claiming millions of lives each year[2]. Primary and secondary exposure to tobacco smoke significantly raises the risk of cancer[5-7], coronary heart disease[8-10], stroke[11, 12], bacterial and viral infections, and has detrimental impacts during pregnancy[13-15].

The detrimental effects of tobacco smoke are not limited to smokers. Non-smokers exposed to second and thirdhand smoke in the environment also show an increased risk for health concerns[4]. Cigarette smoke is composed of thousands of chemicals, of which many are volatile, carcinogenic, and cause DNA damage[16-18]. These chemicals can reside in the environment until inhaled by non-smokers to continue causing devastating health impacts. Secondhand smoke (SHS) is a mixture of what is exhaled by the smoker and what

 doi:10.20944/preprints202205.0141.v1

is emitted by the burning tobacco product, whereas thirdhand smoke (THS) is the residue that accumulates from SHS deposited onto surfaces and can exist in the environment for a long time.

E-cigarettes, also known as electronic nicotine delivery systems (ENDS), were first introduced and marketed as a healthier alternative to cigarettes and to help with smoking cessation[19]. However, new dangers continue to present themselves as ENDS use continues to rise among young adults and adolescents[20]. E-cigarette liquid (e-liquid) is composed of various agents, typically a solvent, nicotine and additional flavoring compounds suspended in a humectant inhaled as anaerosol[21]. Despite the original intention to reduce the detrimental impacts of tobacco smoking, ENDS are largely unregulated and pose increasing concerns as they are proving to be toxic for neonates[22, 23], vascular health[24, 25], respiratory health[26], and damaging to the oral cavity[27].

This paper discusses the pathophysiological mechanisms by which toxicants from tobacco smoke and ENDS negatively impact mitochondrial function. Mitochondria maintain cell metabolism, regulate major pathways and dictate cellular and extracellular responses. As central organelles that meet cell's most energy demands, mitochondria regulate oxidative stress, cell proliferation, and inflammation. Most radicals and reactive oxidative species found within cells are produced and scavenged by mitochondria. Mitochondria also play a vital role in redox signaling[28], cell cycle regulation, differentiation, DNA exchange between cells to restore function, and apoptosis. As a result of their critical role in cell survival and proliferation, mitochondria are often an organelle of interest when studying cellular mechanisms impacting multiple disease processes. Furthermore, mitochondrial susceptibility to damage by exposure to toxicants justifies investigating the impact of environmental chemicals on the organelle[29]. The effects of inhaled toxicants can include inhibition of ATP synthesis due to uncoupling of oxidative phosphorylation[30], increased oxidative mitochondrial damage[31], and mitochondria-initiated apoptosis[32].

Disruptions in mitochondrial function can result from impaired morphology, disruptions in fusion and fission events, increased oxidative stress, or even mutations within the mitochondrial DNA (mtDNA). Impaired mitochondrial morphology and function are implicated in the pathogenesis of pulmonary[33], neurodegenerative[34], diabetic kidney diseases[35], can be pro-tumorigenic[36], and can potentiate inflammatory responses[37]. Mitochondrial DNA, a circular chromosome found within the organelle maintained by nuclear-encoded proteins[38], is another component that can contribute to disease if damaged[39]. Mutations in mitochondrial DNA are responsible for numerous diseases such as optic atrophy[40], mitochondrial myopathy[41], and diabetes mellitus[42].

1. The Mitochondrial Fusion and Fission Machinery

The mitochondria's dynamic and healthy nature is dependent on inter-organelle crosstalk and key processes, including mitochondrial fusion, fission, mitoptosis, and mitophagy[43-46]. The joint forces of mitochondrial fusion and fission, maintained by dynamins and mitofusins, support robust and optimal network morphology[30, 47, 48], and respond to physiological conditions[49]. On the contrary, an imbalance in the proteins determining mitochondrial dynamics can aggravate type 2 diabetes[50], neurodegenerative diseases[51, 52], and can be embryonically lethal[53, 54].

Defective mitochondria are disposed of through mitochondrial fission[55], whereas mtDNA exchange and rescue of damaged mitochondria can happen through fusion[56]. The dynamin superfamily contains a variety of ubiquitous GTPases involving multiple processes including regulating mitochondrial fission. Dynamin-related protein 1 (Drp1) is known to play a critical role in mitochondrial homeostasis by forming fission rings to eliminate damaged parts of the mitochondrial membrane. Drp1 binding requires the presence of different proteins, including Mitochondrial Fission 1 Protein (FIS1) on the outer mitochondrial membrane, mitochondrial dynamics proteins 49kDa (MiD49), 51kDa (MiD51)[57], and mitochondrial fission factor (MFF)[58]. Mitochondrial fission is also dependent on the presence of another class of GTPases known as mitofusins[59]. Mitofusins 1 and

 doi:10.20944/preprints202205.0141.v1

2, located at the outer mitochondrial membrane, reciprocally interact with Drp1 to enable fission[60], support proper embryological development and enhance mitochondrial cooperation to prevent respiratory dysfunction[61]. The mitochondrial dynamin-like GTPase, Optic atrophy 1 (OPA1), is located on the inner mitochondrial membrane (IMM) and induces fusion when there is a loss of membrane potential, therefore strongly linking it to oxidative phosphorylation (OXPHOS)[62]. We demonstrated mitochondrial fusion and fission in cartoon Figure 1.

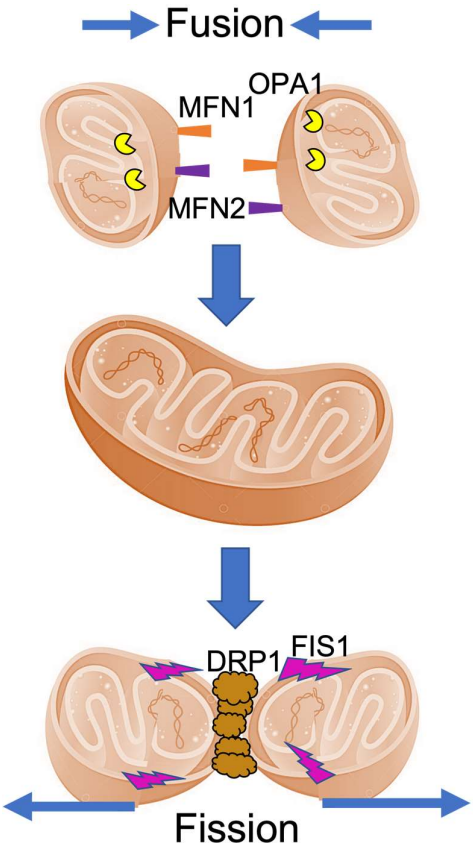

Figure 1. Schematic cartoon of mitochondrial fusion and fission. Mitochondrial fusion occurs when two mitochondria fuse together, whereas fission occurs when one mitochondrion splits into two. Fusion is coordinated on the OMM by the mitofusins (MFN1 and MFN2), and on the IMM by optic atrophy 1 (OPA1). In fission, FIS1 and drp1 are largely involved.

Apart from fusion and fission, mitophagy is another critical process that degrades mitochondria through autophagy. PTEN-induced putative kinase 1 (Pink1), present on the outer mitochondrial membrane, initiates the clearance of defective mitochondria through mitophagy, which is a critical process often seen impaired in various pathological conditions[63-65]. Pink1 works with Parkin, an E3 ubiquitin ligase, to degrade Mfn2, consequently clearing out the defective mitochondria. If any part of Pink1/Parkin machinery is impaired, dysfunctional mitochondria will not be eliminated, further aggravating pathophysiological processes[63]. In this way, mitophagy is quintessential to protecting optimal mitochondrial form and function and operates on multi-tiered processes[66, 67]. Failure to initiate mitophagy or reduction in its process promotes mitochondrial oxidative stress. This failure sets off a cascade of imbalanced signaling pathways, which can accelerate disease processes, including neurodegenerative and cardiovascular conditions[68-70].

1. Cigarette Smoke and E-cigarettes Vape Extraction

 doi:10.20944/preprints202205.0141.v1

In vitro effects of cigarette smoke and e-cigarette vapor are best studied through cell culture media, and consequentially various systems have been developed over time to accommodate studies conducted in larger or small chambers. We will quickly review two examples of how extraction systems are set up. SV Teague et al. developed a method for smaller chambers that is specifically useful for maintaining consistent levels of total suspended particles to replicate relevant environmental conditions of smoke exposure better[71]. Generating smoke requires a system to have both cigarette holding and lighting devices. A metered puff controller can then operate the smoke puffs through a flow machine. Afterward, the chimneys in the conditioning chamber dilute the smoke puffs, and the delivery system distributes smoke to each chamber containing animal or cell-culture sample. Abouassali O et al. extracted e-vapor in cell culture media to study the in vitro toxicity of flavored e-liquids[72]. A 10-cm × 10-cm × 7-cm chamber was made with a bottom opening fitted for the vaping device's mouthpiece. The chamber contained inlet and outlet openings on the top of the lid. The inlet tube received air through an air pump connected to a flow meter, whereas the outlet tube delivered the e-vapor to the cell culture media. Regulating the vacuum connected to flow meter enabled control of puff size and lead to bubbling of the vapor into the cell culture medium[72]. The bubbling of cigarette smoke and e-cigarettes vapor extraction into cell culture media is shown Figure 2.

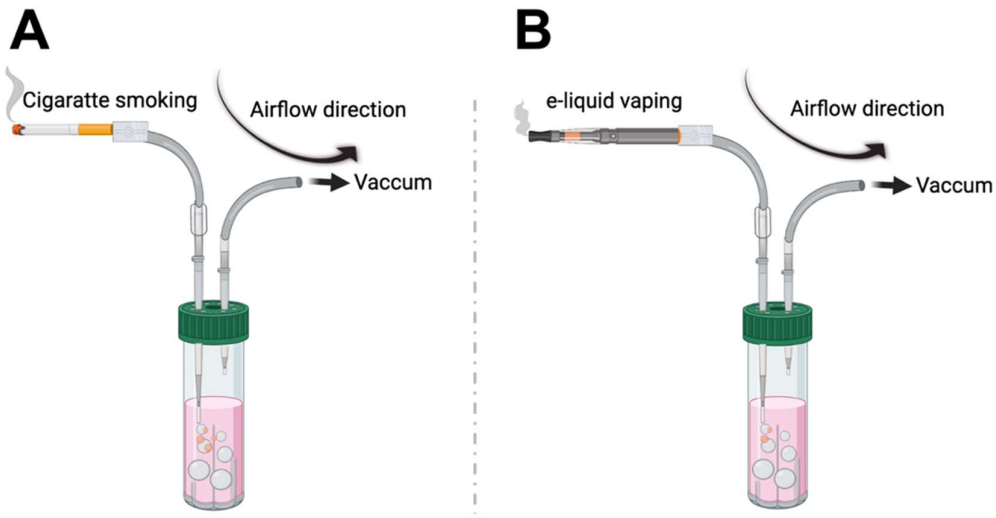

Figure 2. Schematic illustration of cigarette smoke extract (A) and e-liquid vaping (B) into cell culture media.

3.1. Cigarette Smoke (CS) and Cigarette Smoke Extract (CSE) Trigger Mitochondrial ROS

Mitochondrial reactive oxygen species (mtROS) are invaluable intermediates of cell signaling pathways. Their production integrates various biochemical processes to sustain cell survival, signaling, and energetics[73]. The four parts of the electron transport chain make ATP and maintain the organelle's electrochemical potential, which signals the proper function and integration of other metabolic and cellular processes. Disruption in one or more of the processes supporting healthy mitochondrial populations and networking has multifaceted implications and can disrupt the organelle's ability to regulate ROS levels. In excess, mtROS can contribute to mitochondrial dysfunction and in many diseases[74-76]. This undeniable influence of mtROS on other signaling pathways is a key mechanism often implicated by inhaled toxicants[33, 77-79].

Wang Z et al. found that CS increased oxidative stress, reduced respiration, and disrupted the balance of mitochondrial fusion and fission, resulting in altered mitochondrial morphology in primary rat lung microvascular endothelial cells (LMVEC). CS shortened and minimized mitochondrial networks resulting in perinuclear accumulation of

 doi:10.20944/preprints202205.0141.v1

damaged mitochondria primarily through increasing mitochondrial fission by decreasing Drp1-S637 and increasing FIS1, Drp1-S616 phosphorylation[80]. Furthermore, CS caused declining Mfn2 in LMVEC and mouse lungs which reduced mitochondrial fusion and caused mitochondrial translocation, and tetramerization[80]. Smoking has been shown to aggravate oxidative DNA damage due to ROS generated from mitochondrial respiration[81] and can increase mtDNA mutations, in the buccal cells of smokers[82].

Cigarette smoke extract (CSE), in vivo, has been shown to increase both oxidative stress and mitochondrial activity in human fetal fibroblast strains, human lung fibroblast HFL-1 and L828, while also reducing mitochondrial membrane potential to increase cell apoptosis[83]. Similar increases in reactive oxidative species in bronchial epithelial cells utilized lipophilic components of CSE to disrupt mitochondrial function[84]. It has been demonstrated that CSE triggers a proadaptive survival mitochondrial hyperfusion in mouse alveolar epithelial cells accompanied by increased levels of MFN2 within 24h of 10% or 20% CSE treatment[85]. These proadaptive mitochondrial morphology changes were accompanied by increased metabolic activity, ATP levels, and mitochondrial superoxide formation[85].

Hara et al. showed that human bronchial epithelial cells underwent mitochondrial fragmentation and mitochondrial ROS production upon exposure to Cigarette smoke extract, which increased the percentage of cellular death[86]. Whole cigarette smoke condensates (WCSC) decreased cell viability in the normal human bronchial epithelial cell line (BEAS-2B) in a dose-dependent manner and disrupted mitochondrial homeostasis by inducing hypoxic conditions[87].

In human airway smooth muscle, CSE increased Drp1 and reduced mfn2 in a concentration-dependent fashion, which induced mitochondrial fragmentation and damaged morphology by reducing mitochondrial branching and branch length[88]. Furthermore, Aravamudan et al. showed how fusion-fission protein disruption could negatively influence ROS production, cell proliferation, and apoptosis in airway diseases such as asthma and chronic obstructive pulmonary disorder (COPD)[88]. Long-term CSE in COPD primary bronchial epithelial cells induced mitochondrial fragmentation and altered morphology by reducing the number of cristae[89]. These changes in morphology were noted alongside increases in oxidative stress markers, OXPHOS proteins, proinflammatory mediators, and expression of fission and fusion markers.

3.2. The Role of Nicotine in Mitochondrial Dysfunction

Nicotine is one of the many thousands of compounds present in cigarette smoke and commonly found in ENDS. Nicotine exerts its effect by activating nicotinic acetylcholine receptors (nAChRs), present throughout the body, and has been shown to modulate mitochondrial dynamics in hippocampal neurons[90], lung cancer[91, 92], and can impact fetal and neonatal development[93].

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 10 May 2022 doi:10.20944/preprints202205.0141.v1

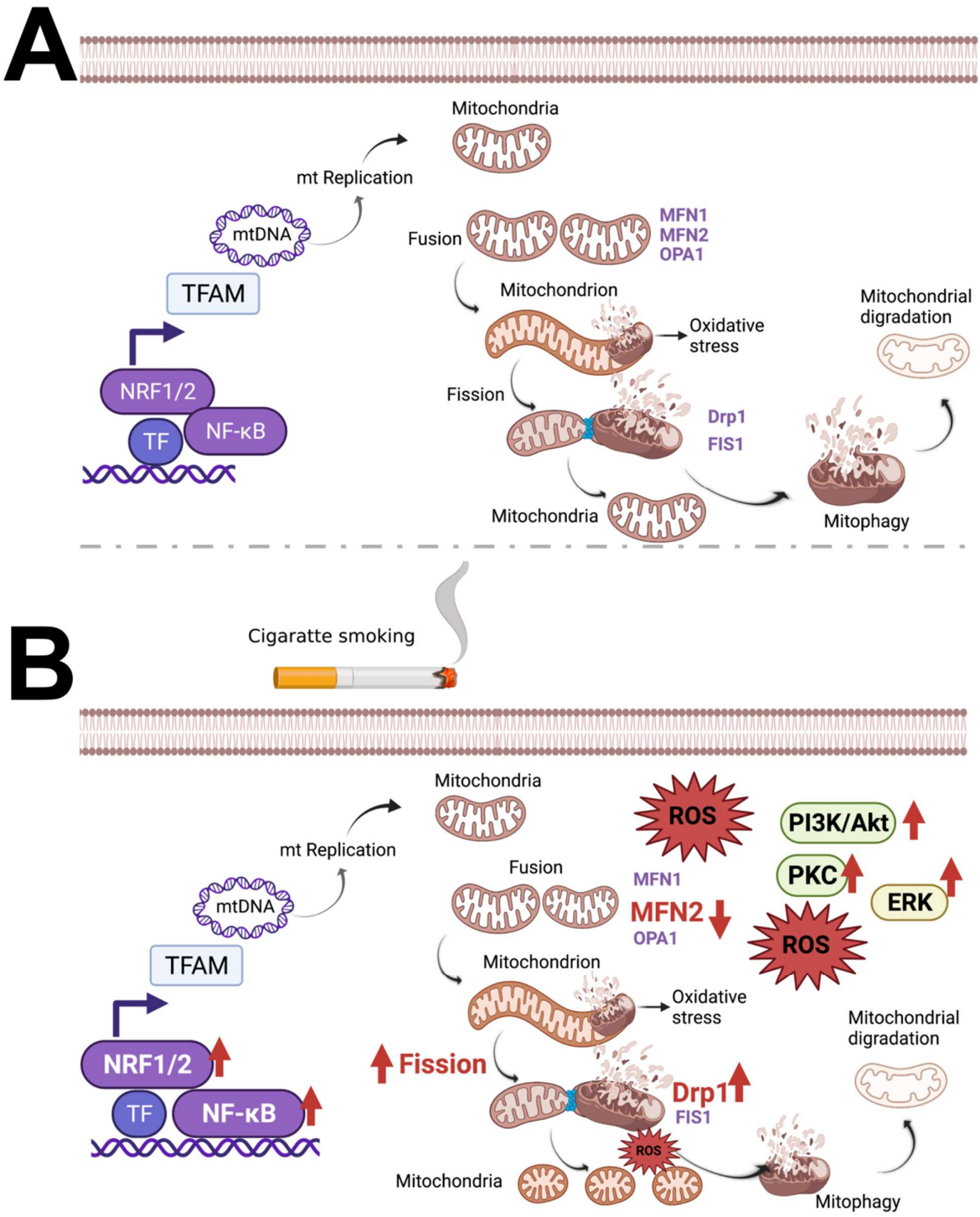

Figure 3. Mitochondrial quality control pathways in healthy cells and CSE treated cells. A. Mitochondrial biogenesis and network regulation rely on key transcription factors and various proteins to maintain the processes needed for the organelle's homeostasis. Mitochondrial transcription factor A (TFAM) acts on mtDNA after being imported into mitochondria and is an essential transcription factor needed to encode mitochondrial proteins. The first of these proteins transcribed include electron transport chain subunits to increase and maintain the appropriate number of mitochondria to sustain oxygen consumption and ATP synthesis. Secondly, fusion proteins (MFN, MFN2, and OPA1) and fission proteins (Drp1 and FIS1) are transcribed to maintain healthy morphology by excising and clearing out damaged portions of the organelle. Mitophagy, a specialized autophagic

 doi:10.20944/preprints202205.0141.v1

pathway, eventually recycles the discarded mitochondrial components[94]. B. In human airway smooth muscle, CS disrupts mitochondrial homeostasis by causing morphological changes and dysfunction. CS-induced mitochondrial fragmentation and damage to networked morphology occurs in a concentration-dependent fashion. CS also increased Drp1 expression, decreased Mfn2, and involved ROS. Furthermore, NF-κB and nuclear erythroid 2-related factor 2 (NRF2) lead to a transcriptional upregulation and increased = activation of extracellular signal-regulated kinase (ERK), phosphatidylinositol 3-kinase (PI3K)/protein kinase B (Akt), and protein kinase C (PKC)[60].

A previously done western blot analysis revealed that nicotine induced mitochondrial fragmentation in human multipotent embryonal carcinoma cell line NT2/D1 by significantly decreasing Mfn1 and Mfn2[95]. Hirata et al. confirmed the mechanism by using a nonselective nAChR antagonist, which effectively blocked nicotine-induced reduction of Mfn1 and Mfn2 protein levels, ATP levels, and mitochondrial fragmentation. Guo et al. proved that in non-small cell lung cancer cells, nicotine-induced activation of hypoxia-inducible factor (HIF)-1α was dependent on mitochondrial-dependent ROS activating downstream Akt and MAPK signaling pathways,[91] figure 3. Maternal nicotine, regardless of cigarette smoking or nicotine replacement therapy, induces oxidative stress targeting the mitochondria as well as β-cell apoptosis in the pancreas, negatively impacting the offspring[93].

3.3. Constituents of fluids used in ENDS are cytotoxic and impair mitochondrial function

As the number of flavors available for e-cigarettes continues to increase, more studies must be done to understand the impact of various flavors and solvents on health[96]. Currently, studies present evidence for the varying toxicity of different flavors, indicating the need to generate a profile of which compounds could be more toxic[72, 97-99]. Some of these flavors and their toxicities will be discussed in this section before further expanding on studies specific to mitochondrial function. Cinnamaldehyde or vanillin-flavored e-vapor was toxic in HL-1 cardiomyocytes and compromised cardiac electrophysiology[72]. H292 human bronchial epithelial cells exposed to strawberry-flavored e-vapor had reduced metabolic activity, reduced cell viability, and increased interleukin release[97]. Cooper et al. demonstrated how vaping-related reinforcement behavior is elevated in male mice when self-administering menthol or green-apple flavored e-liquid compared to no flavor e-liquids[98]. Farnesol, a component of green apple flavor, has been shown to significantly increase nicotine-reward related behavior by altering baseline firing of GABA neurons and upregulating nAChR function, especially in male mice[99]. Interestingly enough, although ENDS are commonly advertised as a safer alternative to cigarette smoking, ECE was found to cause cardiomyocyte toxicities and generate oxidative stress similar to CSE[100]. Jabba et al. demonstrated that solvent adducts of reactive flavor aldehydes are more cytotoxic on lung epithelial cells (BEAS-2B and A549) than their parent aldehydes due to the rapid chemical reaction they undergo with e-liquid solvents, propylene glycol, and vegetable glycerol (PG/VG). Furthermore, these reactive flavor aldehydes reduced ATP synthesis, inhibited mitochondrial function, and were more cytotoxic than their parent aldehydes[101]. Williams et al., demonstrated that MTT assay marked cytotoxicity of two potent chemical toxins in EC solvents, Selenium and Arsenic. Both chemicals inhibited mitochondrial reductases in BEAS-2B cells and proved toxic for pulmonary fibroblasts, whereas selenium increased superoxide production in mitochondria[102].

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 10 May 2022 doi:10.20944/preprints202205.0141.v1

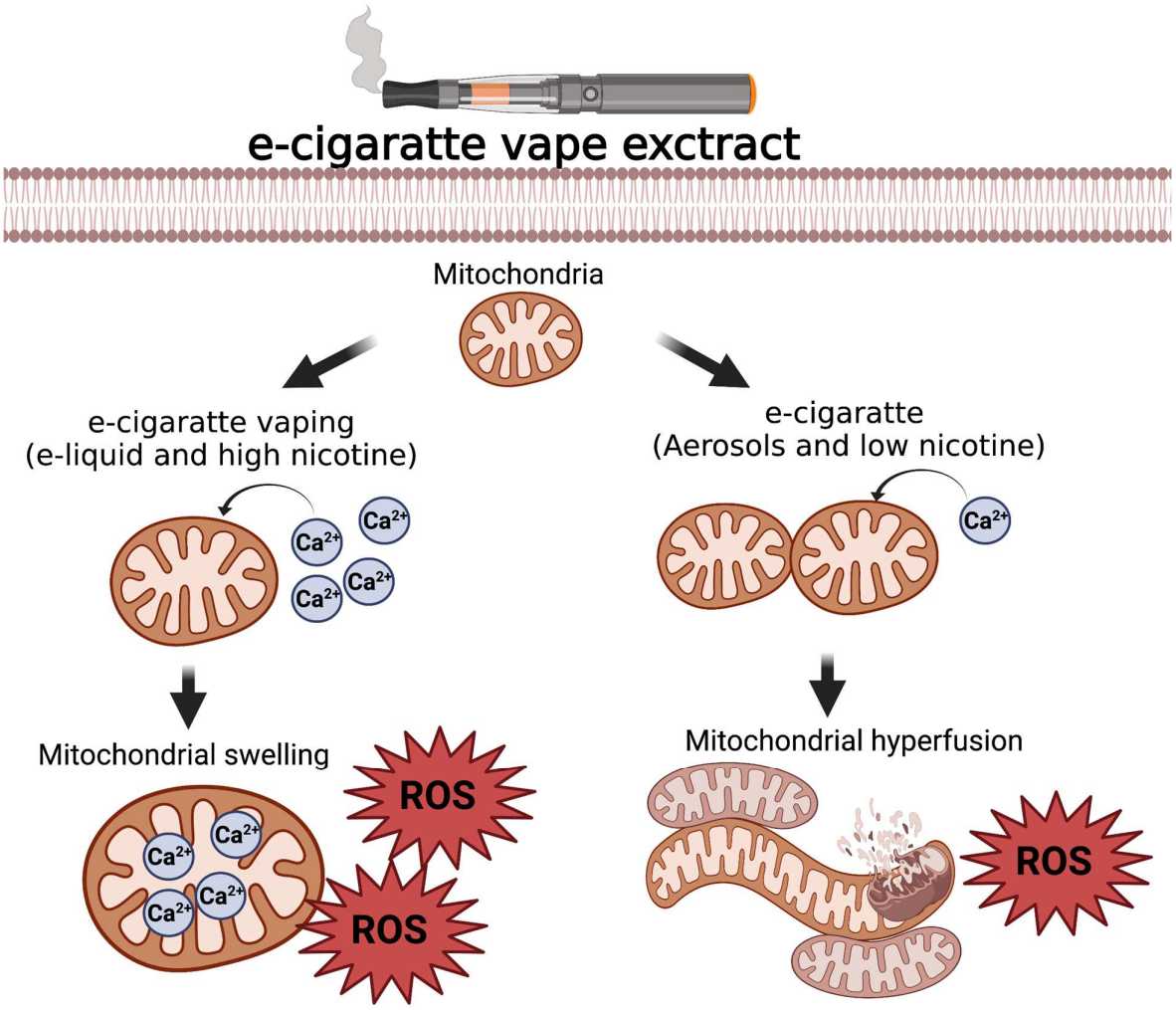

Figure 4. The effects of EC vaping on mitochondrial function. The EC vaping with high concertation of nicotine causes mitochondrial swelling associated with the mitochondrial calcium overload and increases ROS. The EC vaping with aerosols and low nicotine concentration causes mitochondrial hyperfusion associated with stress and elevates the ROS.

4. Maternal Health

The dangers of smoking during pregnancy have long been elucidated, and smoking cessation is advised for the duration of pregnancy. If smoking cessation is impossible, replacing cigarettes with EC is generally advised. Shifting to EC has shown benefits in pregnancy such as restored hepatic lipid metabolism[103]; however, ENDS still pose cytotoxic if used during pregnancy[104]. A previous assessment of in vitro and animal models highlighted the multifaceted mechanisms through which ENDS impacted pre and postnatal brain development[105]. Female Balb/c mice exposed to e-vapor, with and without nicotine, for six weeks before mating showed detrimental changes along with their offspring[106]. EC aerosols and aerosols containing copper nanoparticles elevated mtROS and impaired the stability of electron transport chain (ETC) complex IV in human lung fibroblasts (HFL-1)[107]. Lerner et al. noted that these mitochondrial changes were associated with increased genotoxicity and increased levels of cytokines IL-8 and IL-6. Nicotine and e-cigarette condensate have been shown to disrupt mitochondria by inhibiting OXPHOS complex III and increasing mtROS[108]. This increased mitochondrial dysregulation in redox

 doi:10.20944/preprints202205.0141.v1

signaling was ensued by inhibition of myofibroblast differentiation critical for proper development in HLF-1, which further impaired wound healing[108]. Zahedi et al. demonstrated that the mechanism behind EC-induced stem cell toxicity is stress-induced mitochondrial hyperfusion (SIMH), which results in a transitory survival, followed by increasing mitochondrial oxidative stress[109]. SIMH is identified as a survival response to the nicotine and is largely present in EC refill fluids called do-it-yourself EC products[109]. They also observed that EC leads to cellular stress and diminishes cellular health in the stem cell population, elevates cellular aging, and develops mitochondriopathies[109]. We illustrated EC effect on mitochondria function in figure 4. Maternal vaping during pregnancy may not be as extensively studied as maternal smoking during pregnancy but is a critical and imperative area to be considered when designing further studies.

5. Thirdhand Smoke

While the health impacts of thirdhand cigarette smoke (THS) are still being studied, it is undeniably a crucial component to address regarding public and environmental health. Although experiments on the hazardous nature of THS are limited, an emerging set of data points to reasons not to exclude THS from future studies assessing risk factors. THS has been shown to cause stress-induced mitochondrial hyperfusion (SIMH) in mouse neural stem cells along with increased mitochondrial membrane potential (MMP), increased ATP levels, increased superoxide production, and increased oxidation of mitochondrial proteins[110]. SIMH can also dysregulate gene regulation and transcription has been shown to reduce mitochondrial fission protein Fis1 expression[110], figure 5. In vivo exposure to THS increased AST, urea, and nuclear respiratory factor-1 (NRF1) levels in a time-dependent manner leading to increased liver dysfunction and mitochondrial dysfunction within the liver[111]. THS exposure has been shown to reduce the liver's antioxidant potential in time-dependent manner. This reduction of liver function was seen with some key changes such as, increased oxidative stress, reduced ATP levels, and increased lactate, indicating mitochondrial dysfunction within the liver[111]. Adhami et al. proposed that the connection between the dysfunctional changes was due to a presence of significantly higher TNF-α levels which could play a role in mitochondrial dysfunction given the cytokine's role in many inflammatory and cell death pathways[111].

 doi:10.20944/preprints202205.0141.v1

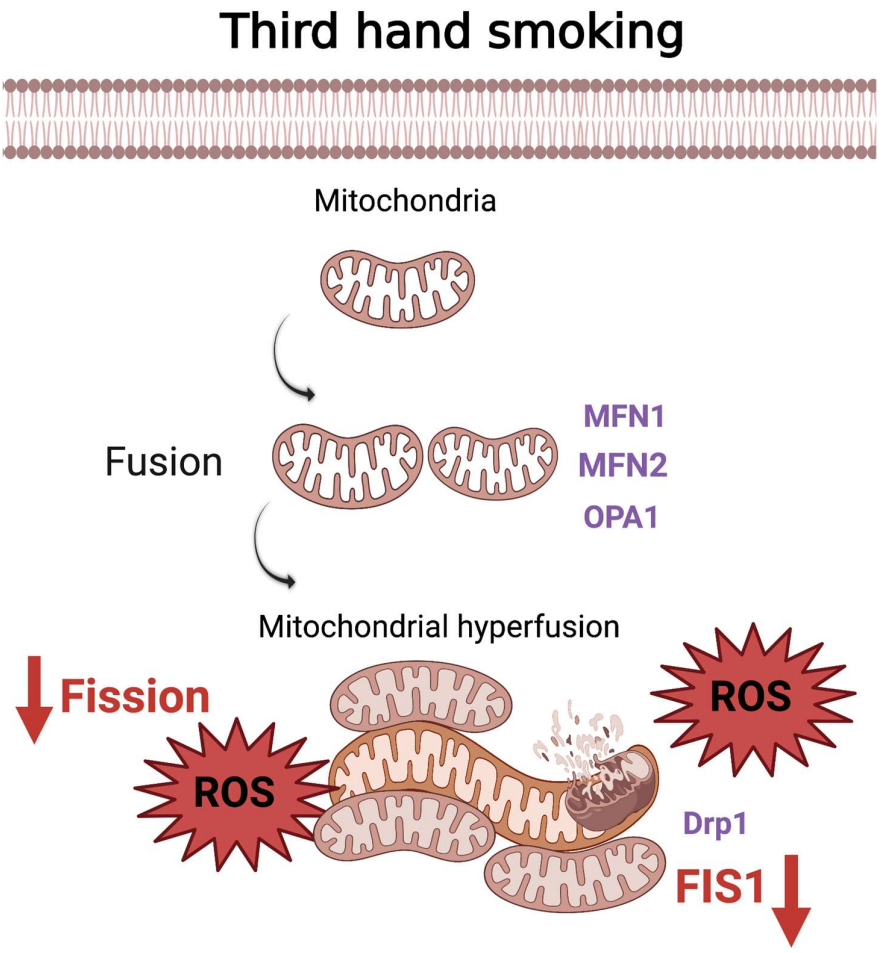

Figure 5. The effect of THS on mitochondria and cell health. THS caused SIMH accompanied by decreased expression of mitochondrial fission protein Fis1 causes the elevation of ROS[110].

Pozuelos et al. conducted a randomized control trial to demonstrate how the acute inhalation of THS increased oxidative stress, mitochondrial membrane potential, ATP production, and decreased permeability of transition of mitochondria and mitochondrial membrane. These changes were accompanied by stress-induced mitochondrial hyperfusion and dysfunction and increased DNA repair mechanisms[112]. Stem cells exposed to THS also showed an increase in SIMH, but upon prolonged exposure, both mitochondrial membrane potential and cell proliferation decreased, ultimately leading to apoptosis of the cell [110].

To summarize, cigarette, e-cigarette smoke is a culprit causing a plethora of devastating and fatal diseases. Although tobacco smoking increases the risk for contracting diseases, psychological and social factors play a key role in maintaining the habit[113]. With an ever-present concern for this public health crisis, toxins in tobacco smoke have an undeniably detrimental effect on mitochondrial health further aggravating the pathophysiological mechanisms of different diseases. Understanding these mechanisms can be helpful in the development of therapeutics. For example, iPSC-MSCs reduced airway inflammation and offered protection against CS-induced mitochondrial oxidative stress, dysfunction, and apoptosis in human ASMCs and in mouse lungs[114].

 doi:10.20944/preprints202205.0141.v1

6. Conclusion

This review highlights how mitochondria damage caused by inhaled intoxicants increases ROS production, apoptosis, reduces respiration, alters mitochondrial membrane potential, and destroys the equilibrium of fission/fusion effects. These detrimental changes contribute to aggravated inflammatory pathways and various disease pathogenesis. Mitochondrial damage responses to smoke vary in a tissue-dependent and concentration-dependent manners, which indicate a need to develop specific studies on understanding the diverse effects and mechanisms which contribute to each unfolding disease process. Understanding these mechanisms can aid in the development of effective interventional and therapeutic modalities.

Furthermore, mitochondrial morphology and health are maintained by the dynamic opposing forces of mitochondrial fusion and fission which are altered by means of various mechanisms involving increased mtROS, increased ATP, dysregulation of key proteins critical, and stress-induced mitochondrial hyperfusion. The intimate connection between mitochondrial morphological changes and dysfunction impairs multiple pathways and alters downstream signaling. Interestingly, these toxic changes vary based on the chemical composition of different e-liquids, therefore further research on the cytotoxicity of aldehyde flavors available for ENDS users is crucial to developing public health guidelines so that specific pathophysiological mechanisms can be elucidated. Although ENDS devices are offered as healthier alternatives to cigarette smoking, it is clear that it is not without risks, especially when concerning maternal health.

Supplementary Materials: Not applicable.

Authors Contribution: Conceptualization: Chidipi. B and Ullah. G. Writing manuscript; Kanithi. M, Junapudi. S, Shah I.S, Alavala. MR, Ullah. G and Chidipi. B.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: please refer to suggested Data Availability Statements in section "MDPI Research Data Policies" at https://www.mdpi.com/ethics.

Funding: This research was funded by NIH-R01 (AG053988) to G.U.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. WHO. Tobacco. 202.
2. WHO. 2020.
3. Grana R, Benowitz N and Glantz SA. E-Cigarettes. *Circulation*. 2014;129:1972-1986.
4. Humans AWGotEoCRt. Tobacco Smoke and Involuntary Smoking. *IARC Monographs on the Evaluation of Carcinogenic Risks to Humans*. 2004;83.
5. Jassem E, Szymanowska A, Siemińska A and Jassem J. [Smoking and lung cancer]. *Pneumonol Alergol Pol*. 2009;77:469-73.
6. Loeb LA, Ernster VL, Warner KE, Abbotts J and Laszlo J. Smoking and lung cancer: an overview. *Cancer research*. 1984;44:5940-58.
7. Macacu A, Autier P, Boniol M and Boyle P. Active and passive smoking and risk of breast cancer: a meta-analysis. *Breast Cancer Res Treat*. 2015;154:213-24.
8. Ambrose JA and Barua RS. The pathophysiology of cigarette smoking and cardiovascular disease: an update. *J Am Coll Cardiol*. 2004;43:1731-7.
9. Leone A, Giannini D, Bellotto C and Balbarini A. Passive smoking and coronary heart disease. *Curr Vasc Pharmacol*. 2004;2:175-82.
10. Rigotti NA and Pasternak RC. Cigarette smoking and coronary heart disease: risks and management. *Cardiology clinics*. 1996;14:51-68.
11. Pan B, Jin X, Jun L, Qiu S, Zheng Q and Pan M. The relationship between smoking and stroke: A meta-analysis. *Medicine (Baltimore)*. 2019;98:e14872.
12. Shah RS and Cole JW. Smoking and stroke: the more you smoke the more you stroke. *Expert review of cardiovascular therapy*. 2010;8:917-932.

 doi:10.20944/preprints202205.0141.v1

13. Li G, Saad S, Oliver BG and Chen H. Heat or Burn? Impacts of Intrauterine Tobacco Smoke and E-Cigarette Vapor Exposure on the Offspring's Health Outcome. *Toxics*. 2018;6.
14. Arcavi L and Benowitz NL. Cigarette Smoking and Infection. *Archives of internal medicine*. 2004;164:2206-2216.
15. Chan YL, Oliver BG and Chen H. What lessons have we learnt about the impact of maternal cigarette smoking from animal models? *Clinical and Experimental Pharmacology and Physiology*. 2020;47:337-344.
16. Talhout R, Schulz T, Florek E, van Benthem J, Wester P and Opperhuizen A. Hazardous compounds in tobacco smoke. *Int J Environ Res Public Health*. 2011;8:613-28.
17. Konstantinou E, Fotopoulou F, Drosos A, Dimakopoulou N, Zagoriti Z, Niarchos A, Makrynioti D, Kouretas D, Farsalinos K, Lagoumintzis G and Poulas K. Tobacco-specific nitrosamines: A literature review. *Food Chem Toxicol*. 2018;118:198-203.
18. Hang B, Sarker AH, Havel C, Saha S, Hazra TK, Schick S, Jacob P, 3rd, Rehan VK, Chenna A, Sharan D, Sleiman M, Destaillats H and Gundel LA. Thirdhand smoke causes DNA damage in human cells. *Mutagenesis*. 2013;28:381-91.
19. Kim S and Oancea SC. Electronic cigarettes may not be a "safer alternative" of conventional cigarettes during pregnancy: evidence from the nationally representative PRAMS data. *BMC Pregnancy Childbirth*. 2020;20:557.
20. Collins L, Glasser AM, Abudayyeh H, Pearson JL and Villanti AC. E-Cigarette Marketing and Communication: How E-Cigarette Companies Market E-Cigarettes and the Public Engages with E-cigarette Information. *Nicotine Tob Res*. 2019;21:14-24.
21. Grana R, Benowitz N and Glantz SA. E-cigarettes: a scientific review. *Circulation*. 2014;129:1972-86.
22. Spindel ER and McEvoy CT. The Role of Nicotine in the Effects of Maternal Smoking during Pregnancy on Lung Development and Childhood Respiratory Disease. Implications for Dangers of E-Cigarettes. *Am J Respir Crit Care Med*. 2016;193:486-94.
23. Kim S and Oancea SC. Electronic cigarettes may not be a “safer alternative” of conventional cigarettes during pregnancy: evidence from the nationally representative PRAMS data. *BMC Pregnancy and Childbirth*. 2020;20:557.
24. Franzen KF, Willig J, Cayo Talavera S, Meusel M, Sayk F, Reppel M, Dalhoff K, Mortensen K and Droemann D. E-cigarettes and cigarettes worsen peripheral and central hemodynamics as well as arterial stiffness: A randomized, double-blinded pilot study. *Vasc Med*. 2018;23:419-425.
25. Fetterman JL and Hamburg NM. A cautionary note on electronic cigarettes and vascular health. *Vasc Med*. 2018;23:426-427.
26. Thirión-Romero I, Pérez-Padilla R, Zabert G and Barrientos-Gutiérrez I. RESPIRATORY IMPACT OF ELECTRONIC CIGARETTES AND "LOW-RISK" TOBACCO. *Rev Invest Clin*. 2019;71:17-27.
27. Ralho A, Coelho A, Ribeiro M, Paula A, Amaro I, Sousa J, Marto C, Ferreira M and Carrilho E. Effects of Electronic Cigarettes on Oral Cavity: A Systematic Review. *J Evid Based Dent Pract*. 2019;19:101318.
28. Collins Y, Chouchani ET, James AM, Menger KE, Cochemé HM and Murphy MP. Mitochondrial redox signalling at a glance. *Journal of cell science*. 2012;125:801-6.
29. Meyer JN, Leung MC, Rooney JP, Sendoel A, Hengartner MO, Kisby GE and Bess AS. Mitochondria as a target of environmental toxicants. *Toxicol Sci*. 2013;134:1-17.
30. Youle RJ and van der Bliek AM. Mitochondrial fission, fusion, and stress. *Science*. 2012;337:1062-5.
31. Jia L, Liu Z, Sun L, Miller SS, Ames BN, Cotman CW and Liu J. Acrolein, a toxicant in cigarette smoke, causes oxidative damage and mitochondrial dysfunction in RPE cells: protection by (R)-alpha-lipoic acid. *Invest Ophthalmol Vis Sci*. 2007;48:339-48.
32. Stridh H, Orrenius S and Hampton MB. Caspase Involvement in the Induction of Apoptosis by the Environmental Toxicants Tributyltin and Triphenyltin. *Toxicology and Applied Pharmacology*. 1999;156:141-146.
33. Tsubouchi K, Araya J and Kuwano K. PINK1-PARK2-mediated mitophagy in COPD and IPF pathogeneses. *Inflammation and Regeneration*. 2018;38:18.
34. Lin MT and Beal MF. Mitochondrial dysfunction and oxidative stress in neurodegenerative diseases. *Nature*. 2006;443:787-95.
35. Wei PZ and Szeto CC. Mitochondrial dysfunction in diabetic kidney disease. *Clin Chim Acta*. 2019;496:108-116.
36. Srinivasan S, Guha M, Kashina A and Avadhani NG. Mitochondrial dysfunction and mitochondrial dynamics-The cancer connection. *Biochim Biophys Acta Bioenerg*. 2017;1858:602-614.
37. West AP. Mitochondrial dysfunction as a trigger of innate immune responses and inflammation. *Toxicology*. 2017;391:54-63.
38. El-Hattab AW, Craigen WJ and Scaglia F. Mitochondrial DNA maintenance defects. *Biochim Biophys Acta Mol Basis Dis*. 2017;1863:1539-1555.
39. Yan C, Duanmu X, Zeng L, Liu B and Song Z. Mitochondrial DNA: Distribution, Mutations, and Elimination. *Cells*. 2019;8.
40. Zanna C, Ghelli A, Porcelli AM, Karbowski M, Youle RJ, Schimpf S, Wissinger B, Pinti M, Cossarizza A, Vidoni S, Valentino ML, Rugolo M and Carelli V. OPA1 mutations associated with dominant optic atrophy impair oxidative phosphorylation and mitochondrial fusion. *Brain*. 2007;131:352-367.
41. Sweeney MG, Bundey S, Brockington M, Poulton KR, Winer JB and Harding AE. Mitochondrial myopathy associated with sudden death in young adults and a novel mutation in the mitochondrial DNA leucine transfer RNA(UUR) gene. *Q J Med*. 1993;86:709-13.
42. Gerbitz KD, Gempel K and Brdiczka D. Mitochondria and diabetes. Genetic, biochemical, and clinical implications of the cellular energy circuit. *Diabetes*. 1996;45:113-26.
43. Venditti P, Di Stefano L and Di Meo S. Mitochondrial metabolism of reactive oxygen species. *Mitochondrion*. 2013;13:71-82.
44. Mishra P and Chan DC. Mitochondrial dynamics and inheritance during cell division, development and disease. *Nat Rev Mol Cell Biol*. 2014;15:634-46.
45. Nunnari J and Suomalainen A. Mitochondria: in sickness and in health. *Cell*. 2012;148:1145-59.
46. Annesley SJ and Fisher PR. Mitochondria in Health and Disease. *Cells*. 2019;8.

47. Archer SL. Mitochondrial fission and fusion in human diseases. *N Engl J Med*. 2014;370:1074.
48. Otera H, Ishihara N and Mihara K. New insights into the function and regulation of mitochondrial fission. *Biochimica et biophysica acta*. 2013;1833:1256-68.
49. Detmer SA and Chan DC. Functions and dysfunctions of mitochondrial dynamics. *Nat Rev Mol Cell Biol*. 2007;8:870-9.
50. Yoon Y, Galloway CA, Jhun BS and Yu T. Mitochondrial dynamics in diabetes. *Antioxidants & redox signaling*. 2011;14:439-57.
51. Olichon A, Guillou E, Delettre C, Landes T, Arnauné-Pelloquin L, Emorine LJ, Mils V, Daloyau M, Hamel C, Amati-Bonneau P, Bonneau D, Reynier P, Lenaers G and Belenguer P. Mitochondrial dynamics and disease, OPA1. *Biochimica et Biophysica Acta (BBA) - Molecular Cell Research*. 2006;1763:500-509.
52. Chen H and Chan DC. Mitochondrial dynamics--fusion, fission, movement, and mitophagy--in neurodegenerative diseases. *Hum Mol Genet*. 2009;18:R169-76.
53. Wakabayashi J, Zhang Z, Wakabayashi N, Tamura Y, Fukaya M, Kensler TW, Iijima M and Sesaki H. The dynamin-related GTPase Drp1 is required for embryonic and brain development in mice. *The Journal of cell biology*. 2009;186:805-16.
54. Chen H, Detmer SA, Ewald AJ, Griffin EE, Fraser SE and Chan DC. Mitofusins Mfn1 and Mfn2 coordinately regulate mitochondrial fusion and are essential for embryonic development. *Journal of Cell Biology*. 2003;160:189-200.
55. Shen Q, Yamano K, Head BP, Kawajiri S, Cheung JT, Wang C, Cho JH, Hattori N, Youle RJ and van der Bliek AM. Mutations in Fis1 disrupt orderly disposal of defective mitochondria. *Mol Biol Cell*. 2014;25:145-59.
56. Chen H, Vermulst M, Wang YE, Chomyn A, Prolla TA, McCaffery JM and Chan DC. Mitochondrial fusion is required for mtDNA stability in skeletal muscle and tolerance of mtDNA mutations. *Cell*. 2010;141:280-9.
57. Palmer CS, Osellame LD, Laine D, Koutsopoulos OS, Frazier AE and Ryan MT. MiD49 and MiD51, new components of the mitochondrial fission machinery. *EMBO Rep*. 2011;12:565-73.
58. Zhang Z, Liu L, Wu S and Xing D. Drp1, Mff, Fis1, and MiD51 are coordinated to mediate mitochondrial fission during UV irradiation-induced apoptosis. *FASEB journal : official publication of the Federation of American Societies for Experimental Biology*. 2016;30:466-76.
59. Eura Y, Ishihara N, Oka T and Mihara K. Identification of a novel protein that regulates mitochondrial fusion by modulating mitofusin (Mfn) protein function. *Journal of cell science*. 2006;119:4913-25.
60. Aravamudan B, Kiel A, Freeman M, Delmotte P, Thompson M, Vassallo R, Sieck GC, Pabelick CM and Prakash YS. Cigarette smoke-induced mitochondrial fragmentation and dysfunction in human airway smooth muscle. *Am J Physiol Lung Cell Mol Physiol*. 2014;306:L840-L854.
61. Chen H, Detmer SA, Ewald AJ, Griffin EE, Fraser SE and Chan DC. Mitofusins Mfn1 and Mfn2 coordinately regulate mitochondrial fusion and are essential for embryonic development. *The Journal of cell biology*. 2003;160:189-200.
62. Song Z, Chen H, Fiket M, Alexander C and Chan DC. OPA1 processing controls mitochondrial fusion and is regulated by mRNA splicing, membrane potential, and Yme1L. *The Journal of cell biology*. 2007;178:749-55.
63. Zhang Y, Liu Q, Li Y, Li C, Zhu Y, Xia F, Xu S and Li W. PTEN-Induced Putative Kinase 1 (PINK1)/Parkin-Mediated Mitophagy Protects PC12 Cells Against Cisplatin-Induced Neurotoxicity. *Med Sci Monit*. 2019;25:8797-8806.
64. Wang N, Zhu P, Huang R, Wang C, Sun L, Lan B, He Y, Zhao H and Gao Y. PINK1: The guard of mitochondria. *Life sciences*. 2020;259:118247.
65. Quinn PMJ, Moreira PI, Ambrósio AF and Alves CH. PINK1/PARKIN signalling in neurodegeneration and neuroinflammation. *Acta Neuropathol Commun*. 2020;8:189.
66. Pickles S, Vigié P and Youle RJ. Mitophagy and Quality Control Mechanisms in Mitochondrial Maintenance. *Current biology : CB*. 2018;28:R170-r185.
67. Lazarou M. Keeping the immune system in check: a role for mitophagy. *Immunol Cell Biol*. 2015;93:3-10.
68. Cai Q and Jeong YY. Mitophagy in Alzheimer's Disease and Other Age-Related Neurodegenerative Diseases. *Cells*. 2020;9.
69. Bravo-San Pedro JM, Kroemer G and Galluzzi L. Autophagy and Mitophagy in Cardiovascular Disease. *Circ Res*. 2017;120:1812-1824.
70. Killackey SA, Philpott DJ and Girardin SE. Mitophagy pathways in health and disease. *The Journal of cell biology*. 2020;219.
71. Teague SV, Pinkerton KE, Goldsmith M, Gebremichael A, Chang S, Jenkins RA and Moneyhun JH. Sidestream Cigarette Smoke Generation and Exposure System for Environmental Tobacco Smoke Studies. *Inhalation Toxicology*. 1994;6:79-93.
72. Abouassali O, Chang M, Chidipi B, Martinez JL, Reiser M, Kanithi M, Soni R, McDonald TV, Herweg B, Saiz J, Calcul L and Noujaim SF. In vitro and in vivo cardiac toxicity of flavored electronic nicotine delivery systems. *American journal of physiology Heart and circulatory physiology*. 2021;320:H133-h143.
73. Hamanaka RB and Chandel NS. Mitochondrial reactive oxygen species regulate cellular signaling and dictate biological outcomes. *Trends Biochem Sci*. 2010;35:505-13.
74. Kussmaul L and Hirst J. The mechanism of superoxide production by NADH:ubiquinone oxidoreductase (complex I) from bovine heart mitochondria. *Proceedings of the National Academy of Sciences of the United States of America*. 2006;103:7607-12.
75. Pryde KR and Hirst J. Superoxide is produced by the reduced flavin in mitochondrial complex I: a single, unified mechanism that applies during both forward and reverse electron transfer. *J Biol Chem*. 2011;286:18056-65.
76. Kudin AP, Bimpong-Buta NY, Vielhaber S, Elger CE and Kunz WS. Characterization of superoxide-producing sites in isolated brain mitochondria. *J Biol Chem*. 2004;279:4127-35.
77. Qin Y, Liu Y, Jiang Y, Mei S, Liu Y, Feng J, Guo L, Du J, Graves DT and Liu Y. Cigarette Smoke Exposure Inhibits Osteoclast Apoptosis via the mtROS Pathway. *J Dent Res*. 2021:220345211009471.

78. Sundar IK, Maremanda KP and Rahman I. Mitochondrial dysfunction is associated with Miro1 reduction in lung epithelial cells by cigarette smoke. *Toxicol Lett*. 2019;317:92-101.
79. Manevski M, Muthumalage T, Devadoss D, Sundar IK, Wang Q, Singh KP, Unwalla HJ, Chand HS and Rahman I. Cellular stress responses and dysfunctional Mitochondrial-cellular senescence, and therapeutics in chronic respiratory diseases. *Redox Biol*. 2020;33:101443-101443.
80. Wang Z, White A, Wang X, Ko J, Choudhary G, Lange T, Rounds S and Lu Q. Mitochondrial Fission Mediated Cigarette Smoke-induced Pulmonary Endothelial Injury. *Am J Respir Cell Mol Biol*. 2020;63:637-651.
81. Pozuelos GL, Kagda MS, Schick S, Girke T, Volz DC and Talbot P. Experimental Acute Exposure to Thirdhand Smoke and Changes in the Human Nasal Epithelial Transcriptome: A Randomized Clinical Trial. *JAMA Network Open*. 2019;2:e196362-e196362.
82. Tan D, Goerlitz DS, Dumitrescu RG, Han D, Seillier-Moiseiwitsch F, Spernak SM, Orden RA, Chen J, Goldman R and Shields PG. Associations between cigarette smoking and mitochondrial DNA abnormalities in buccal cells. *Carcinogenesis*. 2008;29:1170-1177.
83. Baglole CJ, Bushinsky SM, Garcia TM, Kode A, Rahman I, Sime PJ and Phipps RP. Differential induction of apoptosis by cigarette smoke extract in primary human lung fibroblast strains: implications for emphysema. *Am J Physiol Lung Cell Mol Physiol*. 2006;291:L19-29.
84. van der Toorn M, Rezayat D, Kauffman HF, Bakker SJ, Gans RO, Koëter GH, Choi AM, van Oosterhout AJ and Slebos DJ. Lipid-soluble components in cigarette smoke induce mitochondrial production of reactive oxygen species in lung epithelial cells. *Am J Physiol Lung Cell Mol Physiol*. 2009;297:L109-14.
85. Ballweg K, Mutze K, Königshoff M, Eickelberg O and Meiners S. Cigarette smoke extract affects mitochondrial function in alveolar epithelial cells. *Am J Physiol Lung Cell Mol Physiol*. 2014;307:L895-907.
86. Hara H, Araya J, Ito S, Kobayashi K, Takasaka N, Yoshii Y, Wakui H, Kojima J, Shimizu K, Numata T, Kawaishi M, Kamiya N, Odaka M, Morikawa T, Kaneko Y, Nakayama K and Kuwano K. Mitochondrial fragmentation in cigarette smoke-induced bronchial epithelial cell senescence. *American Journal of Physiology-Lung Cellular and Molecular Physiology*. 2013;305:L737-L746.
87. Park EJ, Park YJ, Lee SJ, Lee K and Yoon C. Whole cigarette smoke condensates induce ferroptosis in human bronchial epithelial cells. *Toxicol Lett*. 2019;303:55-66.
88. Aravamudan B, Kiel A, Freeman M, Delmotte P, Thompson M, Vassallo R, Sieck GC, Pabelick CM and Prakash YS. Cigarette smoke-induced mitochondrial fragmentation and dysfunction in human airway smooth muscle. *American Journal of Physiology-Lung Cellular and Molecular Physiology*. 2014;306:L840-L854.
89. Hoffmann RF, Zarrintan S, Brandenburg SM, Kol A, de Bruin HG, Jafari S, Dijk F, Kalicharan D, Kelders M, Gosker HR, Ten Hacken NH, van der Want JJ, van Oosterhout AJ and Heijink IH. Prolonged cigarette smoke exposure alters mitochondrial structure and function in airway epithelial cells. *Respir Res*. 2013;14:97.
90. Godoy JA, Valdivieso AG and Inestrosa NC. Nicotine Modulates Mitochondrial Dynamics in Hippocampal Neurons. *Mol Neurobiol*. 2018;55:8965-8977.
91. Guo L, Li L, Wang W, Pan Z, Zhou Q and Wu Z. Mitochondrial reactive oxygen species mediates nicotine-induced hypoxia-inducible factor-1α expression in human non-small cell lung cancer cells. *Biochimica et biophysica acta*. 2012;1822:852-61.
92. Chernyavsky AI, Shchepotin IB, Galitovkiy V and Grando SA. Mechanisms of tumor-promoting activities of nicotine in lung cancer: synergistic effects of cell membrane and mitochondrial nicotinic acetylcholine receptors. *BMC cancer*. 2015;15:152.
93. Bruin JE, Petre MA, Lehman MA, Raha S, Gerstein HC, Morrison KM and Holloway AC. Maternal nicotine exposure increases oxidative stress in the offspring. *Free radical biology & medicine*. 2008;44:1919-25.
94. Picca A, Mankowski RT, Burman JL, Donisi L, Kim JS, Marzetti E and Leeuwenburgh C. Mitochondrial quality control mechanisms as molecular targets in cardiac ageing. *Nature reviews Cardiology*. 2018;15:543-554.
95. Hirata N, Yamada S, Asanagi M, Sekino Y and Kanda Y. Nicotine induces mitochondrial fission through mitofusin degradation in human multipotent embryonic carcinoma cells. *Biochemical and biophysical research communications*. 2016;470:300-305.
96. Zhu SH, Sun JY, Bonnevie E, Cummins SE, Gamst A, Yin L and Lee M. Four hundred and sixty brands of e-cigarettes and counting: implications for product regulation. *Tob Control*. 2014;23 Suppl 3:iii3-9.
97. Leigh NJ, Lawton RI, Hershberger PA and Goniewicz ML. Flavourings significantly affect inhalation toxicity of aerosol generated from electronic nicotine delivery systems (ENDS). *Tob Control*. 2016;25:ii81-ii87.
98. Cooper SY, Akers AT and Henderson BJ. Flavors Enhance Nicotine Vapor Self-administration in Male Mice. *Nicotine Tob Res*. 2021;23:566-572.
99. Avelar AJ, Akers AT, Baumgard ZJ, Cooper SY, Casinelli GP and Henderson BJ. Why flavored vape products may be attractive: Green apple tobacco flavor elicits reward-related behavior, upregulates nAChRs on VTA dopamine neurons, and alters midbrain dopamine and GABA neuron function. *Neuropharmacology*. 2019;158:107729.
100. Basma H, Tatineni S, Dhar K, Qiu F, Rennard S and Lowes BD. Electronic cigarette extract induced toxic effect in iPS-derived cardiomyocytes. *BMC Cardiovascular Disorders*. 2020;20:357.
101. Jabba SV, Diaz AN, Erythropel HC, Zimmerman JB and Jordt S-E. Chemical Adducts of Reactive Flavor Aldehydes Formed in E-Cigarette Liquids Are Cytotoxic and Inhibit Mitochondrial Function in Respiratory Epithelial Cells. *Nicotine & Tobacco Research*. 2020;22:S25-S34.
102. Williams M, Ventura J, Loza A, Wang Y and Talbot P. Chemical Elements in Electronic Cigarette Solvents and Aerosols Inhibit Mitochondrial Reductases and Induce Oxidative Stress. *Nicotine Tob Res*. 2020;22:S14-s24.

103. Li G, Chan YL, Wang B, Saad S, Oliver BG and Chen H. Replacing smoking with vaping during pregnancy: Impacts on metabolic health in mice. *Reprod Toxicol*. 2020;96:293-299.
104. Northrup TF, Klawans MR, Villarreal YR, Abramovici A, Suter MA, Mastrobattista JM, Moreno CA, Aagaard KM and Stotts AL. Family Physicians' Perceived Prevalence, Safety, and Screening for Cigarettes, Marijuana, and Electronic-Nicotine Delivery Systems (ENDS) Use during Pregnancy. *J Am Board Fam Med*. 2017;30:743-757.
105. Sailer S, Sebastiani G, Andreu-Férnández V and García-Algar O. Impact of Nicotine Replacement and Electronic Nicotine Delivery Systems on Fetal Brain Development. *Int J Environ Res Public Health*. 2019;16.
106. Li G, Chan YL, Wang B, Saad S, George J, Oliver BG and Chen H. E-cigarettes damage the liver and alter nutrient metabolism in pregnant mice and their offspring. *Annals of the New York Academy of Sciences*. 2020;1475:64-77.
107. Lerner CA, Rutagarama P, Ahmad T, Sundar IK, Elder A and Rahman I. Electronic cigarette aerosols and copper nanoparticles induce mitochondrial stress and promote DNA fragmentation in lung fibroblasts. *Biochemical and biophysical research communications*. 2016;477:620-625.
108. Lei W, Lerner C, Sundar IK and Rahman I. Myofibroblast differentiation and its functional properties are inhibited by nicotine and e-cigarette via mitochondrial OXPHOS complex III. *Scientific reports*. 2017;7:43213.
109. Zahedi A, Phandthong R, Chaili A, Leung S, Omaiye E and Talbot P. Mitochondrial Stress Response in Neural Stem Cells Exposed to Electronic Cigarettes. *iScience*. 2019;16:250-269.
110. Bahl V, Johnson K, Phandthong R, Zahedi A, Schick SF and Talbot P. From the Cover: Thirdhand Cigarette Smoke Causes Stress-Induced Mitochondrial Hyperfusion and Alters the Transcriptional Profile of Stem Cells. *Toxicol Sci*. 2016;153:55-69.
111. Adhami N, Chen Y and Martins-Green M. Biomarkers of disease can be detected in mice as early as 4 weeks after initiation of exposure to third-hand smoke levels equivalent to those found in homes of smokers. *Clinical science*. 2017;131:2409-2426.
112. Pozuelos GL, Kagda MS, Schick S, Girke T, Volz DC and Talbot P. Experimental Acute Exposure to Thirdhand Smoke and Changes in the Human Nasal Epithelial Transcriptome: A Randomized Clinical Trial. *JAMA Netw Open*. 2019;2:e196362.
113. West R. Tobacco smoking: Health impact, prevalence, correlates and interventions. *Psychology & Health*. 2017;32:1018-1036.
114. Li X, Michaeloudes C, Zhang Y, Wiegman CH, Adcock IM, Lian Q, Mak JCW, Bhavsar PK and Chung KF. Mesenchymal stem cells alleviate oxidative stress-induced mitochondrial dysfunction in the airways. *J Allergy Clin Immunol*. 2018;141:1634-1645.e5.