The cardiovascular implication for exercise intolerance and dyspnoea in electronic cigarettes smokers.

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List of abbreviations

ANOVA	Analysis of variance
AT	Anaerobic threshold
BLa	Blood lactate
BMI	Body mass index
cmH_2O	Centimetres of water
CO	Carbon monoxide
COPD	Chronic obstructive pulmonary disease
CPET	Cardiopulmonary exercise test
EC	Electronic cigarette users
EELV	End-expiratory lung volume
EILV	End-inspiratory lung volume
eNOS	Endothelial nitric oxide synthase
F_b	Breathing frequency
$FEF_{25\%–75\%}$	Forced mid-expiratory flow
$FEF_{50\%}$	Forced expiratory flow at 50% FVC
$FEF_{75\%.}$	Forced expiratory flow at 75% FVC
FEV_1	Forced expiratory volume in the first second
FEV_1/FVC	Ratio of forced expiratory volume in the first second to the forced vital capacity
FMD	Flow-mediated dilation
FVC	Forced vital capacity

HR	Heart rate
HR_{max}	Maximum heart rate
IC	Inspiratory capacity
ICAM-1	Intercellular adhesion molecule
km	kilometre
L/min	Litres per minute
LDL	Lipoprotein lipase
MEF	Maximal expiratory flow
MEP	Maximal expiratory pressure
Mins	Minutes
MIP	Maximal inspiratory pressure
ml/kg/min	Millilitres per minute per kilogram of body weight
mmHg	Millimetres of mercury
mmol/L	Millimoles per litre
MPO	Myeloperoxidase
MVV	Maximal voluntary ventilation
NO	Nitric oxide
NS	Non-smokers
O_2 pulse	Ratio of oxygen consumption to heart rate
$PaCO_2$	Partial pressure of carbon dioxide in the arterial blood
PEF	Peak expiratory flow
$PetCO_2$	Partial pressure of end-tidal carbon dioxide
$\dot{Q}$	Cardiac output
RER	Respiratory exchange ratio

ROS	Reactive oxygen species
Rpm	Revolutions per minute
SD	Standard deviation
SE	Standard error
TC	Tobacco cigarette smokers
$\dot{V}CO_2$	Rate of carbon dioxide production
$\dot{V}E$	Minute ventilation
$\dot{V}e/\dot{V}co_2$	Ventilatory equivalent for carbon dioxide
VEGF	Vascular endothelial growth factor
$\dot{V}O_2$	Oxygen consumption
$\dot{V}O_{2\ max}$	Maximum rate of oxygen consumption
$\dot{V}o_{2\ peak}$	The peak oxygen consumption at the end of cardiopulmonary exercise test
V_T	Tidal volume
W	Watts

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Abstract

Background: Electronic cigarettes (EC) have been proposed as a safe alternative to tobacco smoking, however, several studies have shown adverse health effects of EC. The chronic implications of EC on cardiorespiratory response and exertional dyspnoea are unknown. Aim: To examine the chronic effects of EC and tobacco cigarettes (TC) on resting vascular function, inflammatory markers, cardiorespiratory responses during exercise and exertional dyspnoea. Methodology: Three groups of young adults (16 each) with normal spirometry (EC users, TC smokers, and never-smokers [NS]) consented to participate in this study following institutional ethics approval. Participants performed forced vital capacity, maximal voluntary ventilation, maximal inspiratory and expiratory pressures manoeuvres to assess lung functions. An incremental cardiopulmonary exercise test (CPET) 20W/2mins on an electronically braked cycle ergometer was performed. Oxygen consumption ($\dot{V}O_2$), heart rate (HR), and ventilation ($\dot{V}_E$) were measured continuously throughout CPET. Blood lactate, dyspnoea, and leg discomfort were recorded at the end of each exercise increment during the CPET. Flow-mediated dilation (FMD%) of the brachial artery was assessed using Doppler ultrasonography.
Results: A one-way ANOVA with Tukey post hoc test was performed. EC and TC groups had a lower peak work rate (WR) compared to NS controls (183.75 ± 30.30; 185.0 ± 34.64 vs. 227.50 ± 43.74 W, respectively; p = 0.006 and p = 0.004). WR ranged from 180-340 W, 140-260 W, 140W-240W in NS, TC and EC respectively. During submaximal exercise, EC and TC groups displayed higher $\dot{V}_E$, higher ratings of dyspnoea and more intense leg fatigue compared to NS controls (e.g. at the highest equivalent submaximal WR of 140 W: $\dot{V}_E$: 62.16 ± 9.48, 64.43 ± 12.34 vs. 53.52 ± 6.88 L/min (p = 0.018; p = 0.011); dyspnoea: 5 ± 2, 6 ± 2 vs. 3 ± 2 Borg units (p =0.033; p <.001); Leg fatigue: 6 ± 2, 7 ± 2 vs. 5 ± 3 Borg units (p = 0.048; p = 0.014), respectively. The TC smokers group displayed lower ventilatory efficiency (higher $\dot{V}_E/\dot{V}CO_2$) and higher blood lactate compared to NS during submaximal exercise (e.g. 140 W, $\dot{V}E/\dot{V}CO_2$: 31.58 ± 5.75 vs. 28.40 ± 2.33, p = 0.036; Blood lactate: 6.54 ± 2.42 vs. 4.29 ± 2.45 mmol/L, p = 0.03, respectively). At peak exercise, breathing frequency (F_b) was lower in EC compared to NS (39.94 ± 7.81 vs. 47.50 ± 9.49 breaths/min, p = 0.037). F_b ranged from (32.26-63.68 breaths/min, 33.69-60.74 breaths/min, and 32.28-61.67 breaths/min) in NS, TC and EC respectively. FMD%

was significantly lower in EC and TC groups compared with NS controls (4.85 ± 2.55, 5.26 ± 3.09 vs. 8.14 ± 3.46 %; p = 0.017, p = 0.040; respectively). FMD ranged from (1.85-15.32 %, 0.76-11.01 % and 1.3-9.56 %) in NS, TC and EC respectively. Conclusion: This study reported impaired vascular and cardiorespiratory responses to exercise and exertional dyspnoea in TC and EC groups in this cohort of young adults with normal spirometry. This suggests that e-cigarettes may impose cardiovascular risks similarly to tobacco smoking.

Key words: Electronic Cigarettes, Flow-mediated Dilation, Exercise, Ventilatory Efficiency, Dyspnoea.

1.Introduction

1.1 Background

Smoking is an aetiological factor in several cardiovascular and respiratory diseases and the most preventable cause of death. There are around 8 million smoking-related deaths per year around the world and approximately 90% of those deaths are preventable if a person stops smoking before the age of 40 years (Jha, et al., 2013). Cardiovascular diseases account for 40% (Ezzati and Lopez 2003), lung cancer is responsible for 20% (Teo et al., 2006) and chronic obstructive pulmonary disease (COPD) is culpable for 20% (Wu and Sin 2011) of smoking-related deaths. There are many toxicants in cigarette smoke, including carbon monoxide (CO), β-unsaturated aldehydes, nitric oxide (NO), and other oxidant gases, all of which have been linked to cardiovascular disease (Deanfield et al.,1986).

The prevalence of smoking in Manchester decreased by 2.7% in 2012 from 24.4% to 21.7% in 2016. Despite this, Manchester still has a higher proportion (21.7%) of its population who smoke compared with England's national average of 15.5% (Jerram and Bendel, 2018). In addition, Manchester has the highest number of premature deaths due to smoking-related illnesses including lung cancer, heart disease and stroke (Jerram and Bendel, 2018). In response, the UK government implemented

plans ‘Towards a smoke-free generation’ with the target of a smoking prevalence of 5% or below by 2030. Manchester has endorsed both the government’s and Public Health England’s method of prescribing e-cigarettes (EC) as a smoking cessation tool (Jerram and Bendel, 2018), after claims from Public Health England that the use of e-cigarettes is 95% healthier than tobacco cigarette (TC) smoking (McNeill et al., 2015). EC have been gaining popularity and there are many different designs and brands of EC with a vast variety of flavours (approximately 7700 different flavours) (Zhu et al., 2014). These include ‘ciga-like’ devices, high voltage EC, refillable tank systems, electronic pipes and cigars and more recently rechargeable and disposable e-cigarettes, which are now the most used devices in current EC users (Leigh et al., 2016; Notley et al., 2024). Although, the addition of EC to standard smoking cessation counselling has resulted in an increased number of smokers who abstained from tobacco for 6 months (Auer et al., 2024). There have been concerns raised about a new generation becoming addicted to nicotine through EC use (Fillon, 2015). Furthermore, there is a paucity of data on the acute and long-term negative cardiovascular impacts of e-cigarettes (Shahandeh et al., 2021).

1.2 Impact of tobacco smoking on vascular function

The main cardiovascular impacts of TC smoking are increased aortic stiffness, blood pressure and vascular endothelial dysfunction (Vlachopoulos et al 2004; Esen et al., 2004). These impacts are significant predictors of atherosclerosis, cardiovascular risk and all-cause increased mortality (Vlachopoulos et al., 2010; Vlachopoulos et al., 2015). Smoking TC has been reported to impair cardiovascular function, due to vascular remodelling (Esen et al., 2004). Chronic TC smoking increases the vascular wall thickness of the intima and media layers, reduces the lumen’s vascular diameter, and reduces the elasticity of the smooth muscle in the vascular walls (Poredoš et al., 1999; Esen et al., 2004). Moreover, TC smoking increases arterial stiffness as a result of endothelial cell apoptosis and proliferation (Esen et al., 2004). Furthermore, TC smoking results in increased oxidative stress which leads to proteosome-dependent degradation of a-tubulin microtubule and the depolymerisation of microtubules (Bernhard et al 2005). This vascular damage alongside sympathetic ganglionic stimulation and activation of a-adrenergic neural reflexes can result in peripheral vasoconstriction (Cryer et al., 1976;). This increased

peripheral resistance alongside smoking-induced myocardial wall stress can lead to hypertension (Cryer et al., 1976; Czernin and Waldherr, 2003). Smoking TC has been reported to impair endothelial function (Esen et al., 2004; Mohammadi et al., 2022). Flow-mediated dilation (FMD) is a significant marker of endothelial function, providing a well-established and early predictor for vascular function and has also been linked to exercise capacity (Inaba, et al., 2010). Esen et al., (2004) investigated brachial artery FMD in twenty long-term smokers (who were smoking an average of 25 cigarettes/day) and 20 age-matched non-smoker (NS) controls. They reported significantly lower FMD in TC smokers compared to NS controls. The mechanisms behind impaired endothelial function are unclear, however, it has been suggested that the chemical components found in particulates of smoke affect endothelial nitric oxide synthase (eNOS) activity and reduce the endothelial production of NO (Barua et al., 2001; Barua et al., 2003).

Further potential mechanisms contributing to endothelial dysfunction in TC smokers include impaired endothelial prostacyclin production and elevated monocyte–endothelial cell adhesion as well as increasing endothelial production of angiotensin II (Reinders et al., 1986; Adams et al., 1997). In addition, nicotine (Mayhan et al., 1999), free radicals and oxidants (Morrow et al., 1995; Hirai et al., 2000; Murohara et al.,1994) found in TC smoke are postulated to be partially responsible for endothelial dysfunction. Chronic TC smoking has also been reported to impair fibrinolysis in plasma which may also contribute to endothelial dysfunction (Simpson et al., 1997). Furthermore, markers of endothelial function including the soluble adhesion molecules von Willebrand factor and thrombomodulin have been identified in TC smokers (Markuljak, et al., 1995) which could also be a contributing factor for endothelial dysfunction (Constans et al., 2006). Likewise, TC smoking has been found to impair cardiac function, with a reduction of the diameter of the coronary arteries, resulting in a decrease in coronary blood flow, and an increase in coronary artery resistance (Quillen et al.,1993). This coronary artery dysfunction coupled with an increased myocardial oxygen demand has been suggested to cause myocardial infarction and cardiac failure (Quillen et al.,1993).

1.3 Electronic cigarettes: Ingredients and cardiovascular implications

There is emerging evidence that EC emit toxicants such as nicotine and carbonyls (Farsalinos et al., 2015; Cheng, 2014). The most common ingredient in EC aerosols is nicotine (Elkalmi et al., 2016). Nicotine is reported to increase the risk of cardiovascular diseases including atherosclerosis, hypertension and heart failure (United States. Public Health Service., 2010; Villarreal et al., 1999; Franzen et al., 2018). Nicotine is a sympathomimetic substance which can result in the increase in cardiac sympathetic nerve activity (Moheimani et al., 2017). Nicotine stimulates the release of norepinephrine from adrenergic neurons and increases epinephrine release from the adrenal glands (United States. Public Health Service., 2010). This results in increased cardiac work by increasing heart rate (HR) (acutely by up to 10-15bpm and an average of 7bpm during the day) and blood pressure (with an acute increase of 5-10 mm Hg) (United States. Public Health Service., 2010).

EC emit other potentially harmful products including carbonyls; which includes aldehydes, such as formaldehyde, acetaldehyde, and acrolein (Goniewicz et al., 2014; Hutzler et al., 2014), which are a by-product of propylene glycol and glycerin (Chand et al., 2020). There is limited literature investigating the impacts of these products on the human cardiovascular system, however, these impacts have been more widely investigated in animals and non-clinical studies (Qasim et al., 2017). Exposure of a formaldehyde solution to a rat's heart have shown acute heart pumping failure (depressed left ventricular end systolic pressure, stroke volume and thus cardiac output ($Q^{\cdot}$) due to the impairment of Ca^{2+} handling in the cardiac excitation–contraction coupling (Tani et al., 1986; Takeshita et al., 2009). EC may also heat flavourings including vanillin, menthol and cinnamaldehyde. These flavourings could be cytoxic to endothelial cells because they have been associated with impaired NO production in human aortic cells (Behar et al., 2014; Clapp and Jaspers., 2017; Fetterman et al., 2018).

1.4 Impact of electronic cigarettes on vascular function

The reports on the chronic impacts of EC on the cardiovascular system are limited and ambiguous compared to the reports of tobacco cigarette smokers. However,

some of the acute effects have been studied. Carnevale et al., (2016) have examined markers that are widely used as indicators of redox state, NO and antioxidant defence as well as FMD (Carnevale et al., 2016). They suggested that the acute use of EC increased oxidative stress with increasing levels of soluble NADPH oxidase 2 (NOX2)-derived peptide and 8-iso-prostaglandin F2α while reducing nitric oxide bioavailability and vitamin E levels.

Other studies highlighted the acute effects of EC use on the cardiovascular system and reported increased systolic blood pressure and an increase in HR temporarily after using an e-cigarette containing nicotine (Franzen et al., 2018; Vlachopoulos et al., 2016). Franzen et al., (2018) suggested that the increased systolic blood pressure and arterial stiffness could be related to nicotine and an increase in circulating and local catecholamines. Some haemodynamic responses to EC use have been explained. Nicotine attaches to nicotinic cholinergic receptors, these receptors are found in the adrenal gland and autonomic ganglia, which leads to an epinephrine and norepinephrine release (Yan and D'Ruiz 2015). This stimulates sympathetic neural mechanisms, alongside the systematic release of catecholamines, causing dose-dependent increases in haemodynamic responses including HR and blood pressure (Benowitz and Fraiman, 2017; Yan and D'Ruiz 2015; Skotsimara et al., 2018). These consequences of e-cigarette use as well as an elevated myocardial demand for oxygen could result in myocardial ischemia (Battista et al., 2013; Benowitz and Fraiman, 2017).

Similarly, to TC smoking, EC use has been reported to cause endothelial dysfunction and lower brachial artery FMD (Mohammadi et al., 2022). The precise mechanisms for endothelial dysfunction in EC users are unclear, however, Mohammadi et al., (2022) linked their findings of lower FMD in EC users of reduced NO and endothelial dysfunction with elevated inflammatory marker levels. In addition, elevated myeloperoxidase (MPO) levels in the sera of EC users have shown to contribute to endothelial dysfunction by inhibiting NO availability and elevating oxidative stress (Hartman and Ford, 2018). Furthermore, the flavourings in EC liquids may have been partially responsible for the reduced NO and inflammation (Mohammadi et al., 2022). Flavourings including vanillin, menthol, and cinnamaldehyde have been

reported to increase IL-6 expression and impair nitric oxide production in human aortic endothelial cells (Fetterman et al., 2018).

The heating of flavourings found in EC are likely to produce toxic compounds including cinnamaldehyde which can be cytoxic to endothelial cells (Behar et al., 2014; Clapp and Jaspers., 2017). This toxicity could be related to decreased endothelium dependent dilation (Carnevale et al., 2016; Olfert et al., 2018). Additionally, endothelial dysfunction could also be associated with EC aerosol extract exposure on human vascular endothelial cells leading to cell apoptosis and necrosis resulting in enhanced DNA fragmentation (Anderson et al., 2016; Putzhammer et al., 2016). These effects were reverted after these cells were treated with antioxidants (Anderson et al., 2016). This endothelial oxidative damage could result in vascular dysfunction and increased cardiovascular disease risk (Qasim et al., 2017). In contrast, Haptonstall et al., (2020) found no differences in FMD% between EC and NS. This was attributed to the sensitivity of FMD and the population's e-cigarette status of light use, as assessed by their low cotinine levels.

1.5 Impact of tobacco smoking on respiratory function

TC smoking impairs pulmonary function (Zamel, et al., 1983). This is shown by decreases in lung function parameters including forced vital capacity (FVC), forced expiratory volume in one second (FEV_1) and decreased forced expiratory flow at 25–75% ($FEF_{25\%–75\%}$) (Kuperman et al., 1973). In addition, tobacco smoking leads to decreases in FEV_1/FVC and $FEF_{25\%–75\%}$ which indicates a potential for developing expiratory airflow limitation (Zamel et al., 1983). Tantisuwat and Thaveeratitham, (2014) reported a lower FVC in young subjects aged 15 to 18 years, with 1-3 years of smoking history. They suggested that this reduction could be the result of the lower maximal expiratory muscle strength in smokers (62.53 ± 22.52 cmH2O) compared to NS (76.35 ± 21.61 cmH2O). Tobacco smoking impacts the respiratory muscles by the impact of free radicals on the vascular system (Ambrose and Barua, 2004), which results in the reduction of blood supply to the respiratory muscles (Tantisuwat and Thaveeratitham, 2014).

Moreover, TC smokers are reported to have a lower lung diffusion capacity which could attribute to a decline in pulmonary capillary blood volume compared to non-smokers (Van Ganse, et al., 1972). In addition, a decline in maximal voluntary ventilation (MVV) has been reported in TC smokers compared to NS. This decline in ventilatory function could be related to smoking-related oxidative stress caused by direct damage to radical species, a smoking-induced inflammatory response and a decline in respiratory muscle strength (Birajdar et al., 2016). Peak expiratory flow rate (PEFR) value has been shown to be lower in tobacco smokers than their predicted values and it has been suggested that this is dose-dependent, with heavier tobacco smokers having a lower PEFR value than light smokers (Karia., 2012).

It is well-established that smoking can cause increased airway resistance, due to changes in the airways including inflammation, mucosa swelling and smooth muscle contraction or due to static factors including the decrease in elastic recoil in lungs and destruction of alveolar attachments and dynamic factors including dynamic compression of the airways (Bohadana et al 2004). Saetta et al., (1994) described the main pathologies of COPD, which include inflammation of the small airways (bronchiolitis) and the destruction of the lung parenchyma (emphysema). They highlighted that these pathologies can result in airflow limitation and that bronchiolitis can contribute to this airflow limitation because the condition can narrow and destroy the lumen as well as constrict the small airways. They also stated that emphysema can contribute to the airflow limitation in TC. It has been suggested that this airflow limitation is present in emphysema because it could reduce the elastic recoil of the lungs (Kim et al., 1991) by destroying lung parenchyma and by the increase in alveolar size (Wright et al., 1987), as well as by the destruction of alveolar attachments reducing the elastic load on the airways (Wright et al., 1987). This airway dysfunction has been related to mechanical time-delays which can be further increased by increases in breathing frequency (F_b) and ventilation ($\dot{V}_E$) during exercise (Elbehairy et al., 2016). These smoking-related impairments may occur over time which trigger physiological mechanisms to compensate for the increased energetic cost and work of the respiratory muscles, and during exercise this includes an increase in $\dot{V}_E$ (Guenette et al., 2014). This increase in $\dot{V}_E$ is also seen in middle age (Sadaka et al., 2021) and elderly smokers (Elbehairy et., 2016) without airflow limitation. This ventilatory inefficiency has been attributed to an increased

physiological dead space (Elbehairy et al., 2015) and a mismatch in the ventilation/perfusion distribution pattern (Jobse et al., 2014).

1.6 Impact of electronic cigarettes on respiratory function

EC use has been reported to impair lung function (Meo et al., 2019). EC use in asymptomatic users with normal spirometry has been reported to contribute to pulmonary vascular dysfunction and ventilation-perfusion mismatch before any alterations in the airways (Kizhakke Puliyakote et al., 2021; Franzen et al., 2018). This impact is similar to the impacts evident in early-stage COPD patients where alterations in ventilation-perfusion inequalities are apparent before airway abnormalities (Barberà et al., 2013). EC use both with and without nicotine causes airway epithelial damage to the bronchial epithelial cells in humans (Chaumont et al., 2019). These changes can also be associated with an increase in airway resistance which can change air flow dynamics especially in smaller airways exposed to e-cigarette vapour. This inflammation and airway resistance has been attributed to ingredients in e-liquids including propylene glycol, formaldehyde and flavouring agents as well as heavy metals affecting the airways (Gerloff et al., 2017; Stockley, et al., 2018). These airway limitations from EC use could have been contributing factors for changes in lung function parameters. Meo et al., (2019) study revealed significant decreases in FEV_1, FEV_1/FVC ratio, $FEF_{50\%}$, $FEF_{75\%}$, $FEF_{25\%–75\%}$, and $FEF_{75\%–85\%}$ in EC compared to NS whereas Vardavas et al., (2012) presented no significant differences in the lung function parameters FVC, FEV_1, and FEV_1/FVC ratio in EC compared to NS. EC use has also been reported to cause a decrease in PEF (Darabseh, 2021).

1.7 Impact of tobacco smoking on exercise capacity

The impacts of TC smoking on exercise capacity have been widely reported (Bernaards et al., 2003; Bolinder et al., 1997; Pirnay, et al., 1971; Kobayashi, et al., 2004; Morton et al., 1985). Smoking TC has been found to impair the cardiorespiratory responses during CPET (Bernaards et al., 2003). Chronic TC smoking causes adaptations including the down-regulation of β-adrenergic receptors (Lauer et al., 1997; Bernaards et al., 2003). This highlighted by a reduced

lymphocyte density and a weakened catecholamine response, indicated by lower ligand binding and decreased cyclic AMP production in response to isoproterenol (Laustiola et al., 1988). Furthermore, lower plasma cyclic AMP levels and reduced fatty acids throughout exercise could indicate β-receptor downregulation in adipose tissue. These adaptations can result in a slower HR increase during exercise (Papathanasiou et al., 2013). This slower heart rate increase in TC can result in a lower ability to achieve the same maximum heart rate (HRmax) (Papathanasiou et al., 2013). It has been postulated that these physiological implications of TC smoking could reduce exercise capacity due to inadequate HR exercise response (Papathanasiou et al., 2013; McDonough and Moffatt, 1999). Although it has been reported that TC smoking can result in a slower HR increase during exercise due to physiological adaptations including receptor down-regulation (Lauer et al., 1997; Bernaards et al., 2003). On the other side, acute TC smoking has showed a higher HR and a lower stroke volume (SV) during submaximal graded upright exercise (Goldbarg et al., 1971). Although no differences were found at rest in SV after smoking compared to the control, SV was significantly lower in all three submaximal graded exercise levels after smoking. Despite sympathomimetic stimulation induced by smoking, the lower SV in smokers suggests an increased inotropic effect on the myocardium. Alternatively, this decrease in SV could be related to a decrease in venous return which results in a compensatory increase in HR. Goldbarg et al., (1971) stated that regardless of the mechanism involved, TC smoking reduces heart efficiency during upright exercise, resulting in a lower SV and increased HR throughout submaximal exercise.

Hirsch et al., (1985) investigated the acute effects of TC smoking in 9 healthy males. Each participant performed an incremental exercise test to volitational exhaustion on two different days. On one day, the test was performed after smoking 3 TC per hour over 5 hours. On the other day, the test was performed without smoking. They reported a lower oxygen (O_2) pulse on the smoking day compared to the non-smoking day (Hirsch et al., 1985). O_2 pulse is associated with the O_2 extracted from the blood delivered to the tissues by each heartbeat, which is equal to the product of SV and arteriovenous O_2 content. Hirsch et al., (1985) suggested that the lower O_2 pulse found in smokers at rest and at matched work rates in their study could be related to the failure of normal vasodilation to the working muscles because they

also found a trend towards a raised systolic pressure on the smoking day. This is further highlighted in Tachmes et al., (1978) study as they reported an increase in HR, and blood pressure directly related to the effects of nicotine in TC.

Ventilatory inefficiency (higher $\dot{V}_E/\dot{V}CO_2$) has been associated with FMD in TC smokers and has been linked to TC smoking-related early pulmonary vasculopathy (Gläser et al., 2011). Higher ventilation and $\dot{V}_E/\dot{V}CO_2$ during submaximal exercise were attributed to increased physiological dead space and ventilation/perfusion mismatch (Sadaka et al., 2021; Barbosa et al., 2017). Elbehairy et al., (2016) study reported no differences in the $\dot{V}_E/\dot{V}CO_2$ in TC smokers without airflow limitation compared with NS (Elbehairy et al., 2016). However, their study showed considerable variability in $\dot{V}_E/\dot{V}CO_2$ among smokers. Additionally, an inverse relationship between $\dot{V}_E/\dot{V}CO_2$ nadir and $\dot{V}O_2$max was found (Elbehairy et al., 2016). This could suggest that smokers with ventilatory inefficiency (indicated by a higher $\dot{V}_E/\dot{V}CO_2$ nadir), tended to have lower $\dot{V}O_2$max and a reduced exercise capacity (Elbehairy et al., 2016). Another potential mechanism for this ventilatory inefficiency could include a lower partial pressure of carbon dioxide ($PaCO_2$) (Naeije., 2014). This decrease in $PaCO_2$ and increase in dead space could reflect impaired neuro-humoral ventilatory control mechanisms (Naeije et al., 2014). This includes increased ventilatory control ‘gain’, which is seen in hyperventilatory conditions including hypoxemia, metabolic acidosis, and increased central neural respiratory drive (Ward, 2021). Furthermore, this increased work of the respiratory muscles resulted in increased inspiratory neural drive to the diaphragm in smokers which could have contributed to increased ratings of perceived dyspnoea (Elbehairy et al., 2016). Dynamic hyperinflation has been reported to cause a decrease in inspiratory muscle strength due to shortening the length of the diaphragmatic muscle fibres (Sinderby et al 2001; O'Donnell et al., 1997; Beck et al., 1998; McCully and Faulkner, 1983). Gauthier et al., (1994) reported that dynamic hyperinflation decreases the pressure-generating capacity of the diaphragm because it impacts the degree to which it is positioned to the rib cage and the lungs, its three-dimensional shape and its length-force properties. Therefore, there is an increased work and oxygen cost of breathing due to accelerated increases in the inspiratory muscle’s elastic and threshold (Calverley and Koulouris., 2005). Moreover, dynamic hyperinflation decreases the ability of tidal volume (V_T) to increase to meet increased exercise

demands (Calverley and Koulouris., 2005). This is because hyperinflation leads to a plateau in V_T during exercise, as ventilation increases. This occurs at a critically low dynamic inspiratory reserve volume (IRV) of 0.5–1.0 L (O'Donnell and Laveneziana, 2006). At this plateau, the ability of V_T to expand further is reduced and V_T becomes fixed at the upper, less compliant end of the respiratory system's sigmoid-shaped pressure-volume (P-V) curve, which results in increased elastic loading of the inspiratory muscles (O'Donnell and Laveneziana, 2006). Consequently, this decreases the ability to meet the heightened ventilatory demands during exercise and an earlier onset of dyspnoea (O'Donnell et al., 2012). As a result, impaired exercise capacity was attributed to dynamic hyperinflation (Frazão et al., 2019; Soffler et al., 2017).

Additionally, a lower anaerobic threshold (AT) as a percentage of $\dot{V}O_2$max has been reported in TC smokers compared to NS (Hirsch et al.,1985). This has been attributed to decreased oxygen delivery to the working muscles which is the result of a decreased $\dot{Q}$ and increased carboxyhaemoglobin (Hirsch et al.,1985). This shift to anaerobic metabolism results in increased blood lactate (BLa) and leg discomfort (LF) in smokers (Huie et al., 1996). These symptoms of increased LF and dyspnoea become intolerable at a lower work rate (WR) in TC smokers which could contribute to a lower $\dot{V}O_2$max and an earlier termination of exercise (Rooks et al., 2011; Soumagne et al., 2020). These impairments alongside increased ventilatory inefficiency can ultimately cause a lower exercise capacity in TC smokers compared to non-smokers (Melliti et al., 2021; Rooks et al., 2011; Soumagne et al., 2020).

1.8 Impact of electronic cigarette use on exercise capacity

Previous literature investigating the impact of e-cigarette use on the cardiorespiratory responses during exercise is limited and ambiguous. The acute effects EC with nicotine include an increase in HR, systolic blood pressure and carbon monoxide (CO) (Franzen et al., 2018). Kizhakke Puliyakote et al., (2021) also suggested that EC can also result in the redistribution of blood flow to the pulmonary capillary beds. However, Polosa et al., (2017) found no changes in HR between EC and NS during exercise. In contrast, a meta-analysis comprising of 11 studies and 283 participants

determined that EC use does increase HR at rest (Skotsimara et al., 2018) similarly to TC smoking but potentially less severely (Yan and D'Ruiz., 2015). This increase in HR has been attributed to nicotine contained in EC by sympathetic neural mechanisms and the systematic release of catecholamines (Benowitz and Fraiman, 2017). A small, unpublished study included 19 EC users, with a minimum of 6 months of EC use, found no difference in $\dot{V}O_2$max compared to non-users. This short period of EC use could be significant when investigating their impacts. Suminski et al (2009) study reported a lower $\dot{V}O_2$max in heavy smokers compared to light smokers (groups depended on the number of packs smoked per year). This could suggest that similarly to TC, $\dot{V}O_2$max could also be dose-dependent in EC, insinuating that e-cigarette use for longer period could impair $\dot{V}O_2$max and other cardiorespiratory responses at submaximal workloads. Therefore, this study assesses the cardiorespiratory responses to exercise and dyspnoea in EC and TC who have used e-cigarettes or smoked tobacco cigarettes for a minimum of 18 months.

Cardiopulmonary exercise testing provides an early indicator of cardiopulmonary impairments (Kailas et al., 2021). Therefore, exercise may be a more sensitive intervention compared to resting measures in showing early cardiopulmonary impairments in young e-cigarette users. Currently, there is no clear consensus to determine the long-term impacts of e-cigarettes on the cardiovascular system and dyspnoea during exercise. Therefore, it is of concern to determine the chronic impacts of e-cigarettes on cardiovascular and respiratory health so that public health policies would be evidence-based.

1.9 Aims:

This cross-sectional study investigates the effects of chronic tobacco smoking and electronic cigarette use in young adults with normal spirometry on vascular function and exercise capacity. This includes the cardiorespiratory responses and dyspnoea during exercise and resting endothelial function as a potential contributor to exercise intolerance.

1.10 Hypothesis

The hypothesis for this study is that cardiorespiratory responses including dyspnoea during exercise will be similarly impaired in TC and EC users compared to NS. It is also postulated that flow-mediated dilation will be lower in TC and EC compared to NS.

To test this hypothesis, $\dot{V}O_2$, HR, WR, dyspnoea, $\dot{V}_E$, $P_{ET}CO_2$, V_T, F_b, $\dot{V}_E/\dot{V}CO_2$, $\dot{V}O_2$/HR, BLa, dyspnoea and LF during CPET and FMD of the brachial artery will be compared between tobacco smokers, electronic cigarette users and non-smokers.

2.Methodology

2.1 Ethics

Full ethical approval was obtained from the Science & Engineering ethics committee at Manchester Metropolitan University on the 03/07/2023 (EthOS reference number: 57005). All procedures performed involving human participants were in fulfilment of Manchester Metropolitan University for the degree of Master of Science (by Research) and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Written consent from each participant was provided and they were informed that taking part in the study was voluntary before participating in the study. Participants were identified by Agata Giles (MRes student) and were recruited from Manchester Metropolitan University or through word of mouth, using tools including participant recruitment posters placed around the university which provided contact details for participants to show their interest as a volunteer. Following this, participants were emailed the participant information sheet so they could give informed consent. Participants could withdraw without justification at any point.

2.2 Subject characteristics

48 participants consented to take part in this study. 3 groups of young adults, age matched (aged 18-30 years) and gender matched (16 each:10 males and 6 females) with normal spirometry (TC, EC, and NS). Inclusion criteria required all participants to have normal spirometry. FVC and FEV_1 must be >80% predicted and FEV_1 / FVC >70%, and both with z-scores >−1.64 from the 2012-Global Lung Function Initiative equations] (Quanjer et al., 2012; Graham et al., 2019).

TC smokers and EC users must have been smoking or using electronic cigarettes for ≥18 months. EC users must use ≥1 EC device/week. The inclusion criteria required all participants to have a normal body mass index (BMI: ≥18.5 kg/m^2 to <35 kg/m^2). The exclusion criteria required all subjects to have no contraindications to exercise or vascular function such as a history of cardiovascular or respiratory disease. Participants were required to not consume caffeine, vape or smoke (or any other form of nicotine) or undergo exercise activities ≥24 hours prior to any testing visit. Tobacco smokers had no history of e-cigarette use. Electronic cigarette users who had a history of tobacco smoking have been excluded from the study. However, one EC user had a short history of tobacco smoking 5 years prior to study.

2.3 Data collection

Subject's height and weight were measured using a stadiometer in cm, and a mechanical column scale in (Kg), respectively. BMI was calculated by dividing weight by height squared (kg/m^2). A blood pressure reading was taken non-invasively from the left brachial artery using an automated sphygmomanometer (Omron M6 comfort, Omron healthcare, Kyoto, Japan).

On day one, participants were asked their subject characteristics including gender, age, smoking history and how many times a week a participant took part in physical activity. Participants physical measurements were taken, including height and weight. Following this, participants had their blood pressure taken. The participants' respiratory muscle strength was then measured, followed by spirometry, participants then completed 10 minutes of rest before a CPET was performed. Every participant's

spirometry and CPET was conducted in the same laboratory in the Institute of Sport at Manchester Metropolitan University.

On day two, flow-mediated dilation was performed in the same laboratory for every participant as the CPET and spirometry in the Institute of Sport at Manchester Metropolitan University. Day one (CPET and spirometry) was always performed first, followed by day two (FMD). All measurements were performed within the same week. Participants had ≥ 48hr rest between each testing day.

Agata Giles performed CPET, spirometry to gain lung function measurements (including forced vital capacity and forced expiratory volume in the first second, maximal inspiratory and expiratory pressure). FMD was performed by Dr Rehan Junejo.

Throughout all testing, room temperature was kept at ~22°C and although not kept constant, humidity was between 29-30%.
Measurements and protocols are described in more detail below.

2.4 Visit 1: Spirometry and respiratory pressure

Maximal inspiratory pressure (MIP) and maximal expiratory pressure (MEP) were measured using Micro spirometry (MicroMPM Spirometer, MD Spiro, Maine, USA) with participant sitting upright with a nose clip. The subjects blew into the micro spirometer and inspiratory or expiratory effort was continued for at least 1 s (Neder et al., 1999). MIP and MEP were repeated three times and the best trial were recorded in cm H_2O (Polkey et al., 1995).

Spirometry was conducted using the Vyntus CPX (Vyaire Medical, Hochberg, Germany) according to the American Thoracic Society Standardisation of Spirometry (Graham et al., 2019). Participants sat with a nose clip and performed two different types of spirometry tests MVV and forced spirometry into a mouthpiece connected to a digital volume transducer (DVT) to assess lung function parameters including FVC, FEV_1, forced expiratory volume in the first second ratio to forced vital capacity (FEV_1/FVC), inspiratory capacity (IC), and MVV.

The FVC manoeuvre was performed according to the American Thoracic Society Standardisation of Spirometry (Graham et al., 2019). Subjects inspired rapidly and maximally (maximal inspiration) and paused for ≥2-s for total lung capacity, they continued to complete expiration (until no more air could be expired and then inspired back to maximal (to maximal lung volume). Three acceptable trials were performed and checked for FEV_1 and FVC repeatability, the highest trial value for FEV_1 and FVC was recorded from the Vyntus.

The MVV manoeuvre was in accordance with guidelines (Neder et al., 2019). MVV is the largest volume of air that is inhaled and exhaled of the lungs during a 12-15-s interval. To achieve this, subjects breathed deeply and rapidly. This was performed with maximal voluntary effort. Following the first three to five breaths which were excluded, the subjects were encouraged to maintain the same volume and frequency. When at least three acceptable trials were obtained, with no more than 10% difference between them, the highest trial value was recorded from the Vyntus (Neder et al., 1999).

2.5 Visit 1: Cardiopulmonary exercise testing (CPET)

Following 5 minutes of rest, and 1 minute warm-up of unloaded cycling, stepwise incremental cardiopulmonary exercise test to volitional exhaustion was performed at 20W/ 2 min on an electronically braked cycle ergometer (Ergoselect 5, Ergoline, Germany). Measurements including $\dot{V}O_2$, $\dot{V}CO_2$, $\dot{V}_E$, $P_{ET}CO_2$, V_T, F_b ,and $\dot{V}_E/\dot{V}CO_2$, were continuously measured on a breath-by-breath basis using the Vyntus CPX system (Vyaire Medical, Hochberg, Germany). HR was continuously measured in beat-by-beat basis using a polar HR monitor (Polar H7 bluetooth, Kempele, Finland). A single-use disposable lancet (Roche Accu-Chek, Basel, Switzerland) was used to pierce the side of the fingertip to gain a drop of blood. This small aliquot of blood was transferred using lactate pro 2 strips to the lactate analyser (Lactate Pro 2, Arkray, Kyoto, Japan), to assess BLa in the last 10 seconds of each incremental 20W stage. Participants maintained a constant cadence of 60 rpm and the test was terminated when the subject was unable to continue or maintain > 60 rpm cadence. The modified 10-point Borg scale (Borg, 1982) was used at the end of each incremental

stage to assess symptoms of ‘breathing discomfort’ (dyspnoea) and leg discomfort (LF).

2.6 Visit 2: FMD

FMD was performed in accordance with the latest international guidelines (Thijssen et al., 2019). The same investigator (Dr Rehan Junejo) performed all FMD tests.

All vascular function for FMD testing was performed by Dr Rehan Junejo, with the majority of testing performed in the morning, and two participants had their measurements taken in the afternoon. This should not have contributed to any differences in FMD between the groups.

FMD of the brachial artery was assessed using Doppler ultrasonography (Terason uSmart 3300, TeraTech, Burlington, MA, USA). Participants lay in a supine position for approximately 10 mins rest before any measurements were taken and an occlusion cuff was placed 5cm distal to the medial epicondyle.
Brachial artery diameter and mean blood flow velocity were measured using a 7.5MHz ultrasound linear probe at rest, during and after the 5 minutes of occlusion. Duplex imaging using the B-mode ultrasound was taken of the brachial artery diameter 10-15cm proximal using a 60° insonation angle. Following 1-min of baseline measurement of the brachial artery diameter and blood flow velocity, the rapid occlusion cuff was inflated ~ 250mmHg for 5 minutes to occlude blood flow from the brachial artery. This inflation level was based on the participant’s mean systolic blood pressure plus either 50 or 100 mmHg always resulted in an average occlusion blood pressure below 250 mmHg. The occlusion period was then terminated by rapid deflation of the occlusion cuff. Brachial artery diameter and blood flow velocity were measured for 3 minutes post-ischemia.

2.7 Data Analyses

Although this study did not measure physical activity formally via a physical activity questionnaire or using an accelerometer, each participant verbally confirmed that they take part in physical activity ≥ once/week. Additionally, the estimated VO_2max value for each subject was calculated using sex-specific equations (Silva et al., 2015). The equation for men and women was calculated as (final workload/body weight) x 10.791+7 and (final workload/body weight) x 9.820 + 7, respectively (Silva et al., 2015). VO_2max (ml/kg/min) as a % of predicted ($\dot{V}O_2$ ml/kg/min- %predicted) was calculated as VO_2max (ml/kg/min)/estimated VO_2max (ml/kg/min)*100. The mean and standard deviation was calculated for $\dot{V}O_2$ (ml/kg/min - %predicted). Average $\dot{V}O_2$ ml/kg/min- %predicted for each smoking status group was ~100%. This highlights that muscle deconditioning was not a limiting factor for exercise impairments found in this study. It also highlights that participants are 'active'.

Lung function parameters including MVV, FEV_1, FEV_1/FVC, IC, FVC were taken from predicted values from Vyntus CPX. These predicted values were based on predicted ranges for age, ethnicity, gender and height from the Global Lung Function Initiative (GLI) (Quanjer et al., 2012). The best trials of MIP and MEP were recorded out of three repeats.
Breath-by-breath measurements taken throughout the CPET were extracted from the Vyntus CPX and analysed in Microsoft Excel as the last 30 s average of each work rate, average of the last 3 minutes at rest, and at 'peak exercise'.

$\dot{V}O_2$max was determined as the average $\dot{V}O_2$ data recorded during the last 30 s of exercise. $\dot{V}O_2$max was attained by the participants achieving the following criteria for maximal effort: A respiratory exchange ratio (RER) > 1.10 as well as performing to > 85% HR_{max} predicted for age (Balady et al., 2010). $\dot{V}O_2$max was reported as absolute values (L/min) and relative to each subject's body mass (ml/kg/min).

The ventilatory anaerobic threshold (VAT) (respiratory compensation point at the lactate threshold, with lactate at approximately 4 mmol/L) was determined by two assessors Agata Giles and Dr Azmy Faisal using the 'v slope' method and confirmed using the ventilatory equivalents (Beaver et al., 1986) and a rapid/distinct change in the inclination of the BLa curve. The v-slope method is the exercise intensity, where the plot of $\dot{V}CO_2$ and $\dot{V}O_2$ intersect and show an increase in the slope above 1.0 (Bland and Altman, 1986). VAT was reported in (L/min) and (ml/kg/min) and reported as a percentage of $\dot{V}O_2$max and peak WR (Hansen and Wasserman., 1984).

A partially automated edge-detection and wall-tracking programme algorithms which works independently of investigator bias (Cardiovascular Suite version 3.4.1; FMD Studio, Pisa, Italy). This programme is a dedicated FMD software with edge-detection capability as well as recording of B-mode ultrasound image sequences. The edge detection and wall tracking software measured continuous changes in brachial artery diameter. The investigator choses a region of interest with optimal image quality. FMD% was calculated as (change in arterial diameter after occlusion release divided by the baseline diameter X 100) [FMD = (Dmax – Dbaseline/Dbaseline) multiplied by 100. The relative diameter change and time to peak diameter change is calculated automatically by the software, free from investigator bias (Thijssen et al., 2019). Ultrasound measurements were taken in accordance with expert protocol guidelines (Thijssen et al., 2019). Mean blood-flow velocity can be estimated by taking half the peak velocity. This approach measures the fastest moving blood cells located in the centre of the vessel (Li et al., 1993). Shear rate was calculated as the brachial artery blood velocity multiplied by 4 and divided by brachial artery diameter (Parker et al., 2009).

2.8 Statistical analysis

A sample size of 16 subjects per group was estimated to detect differences in FMD and exercise capacity (effect sizes of 0.89 and 1.43, respectively) between the groups using an ANOVA test with a power of 80% and a significance level of 0.05 (Faul et al. 2007). A Shapiro-Wilk test was performed to test for normal distribution of data (p>0.05). A one-way ANOVA with Tukey post hoc test was performed using SPSS (IBM Statistics 29) to determine any differences between the three groups in

variables from the CPET, FMD% and spirometry data. $P < 0.05$ determines the statistical difference. Unless stated otherwise, all values are presented as mean ±SD. For data which showed significance during ‘comparable’ iso-submaximal workloads, 140 W was used for displaying the results because 140 W was the highest equivalent workload reached by every participant in each group.

3. Results

3.1 Subject characteristics

Groups had comparable ages, height and BMI (Table 1) and were identically matched for gender. TC group had a longer duration of smoking history compared to EC (5.8 ± 6 years, vs. 3 ± 2.2 years, p <0.001). TC group had a smoking history index of 7 ± 3 and EC group disposable-cigarettes use weekly is 2 ± 1.

Table.1 Baseline characteristics, including demographic and anthropometric data grouped by smoking status (NS, TC and EC)

Variable	Non-smokers	Electronic smokers	Tobacco cigarette smokers	*P* value
Male: female	10:6	10:6	10:6	-
Age, years	23 ± 3	22 ± 2	23 ± 3	0.674
Height, cm	174.5 ± 8.1	174.2 ± 7.5	176.8 ± 7.8	0.583
Body mass index (k/m^2)	22.7 ± 2.1	23.6 ± 3.6	23.7 ± 3.1	0.628
Smoking duration (years)	0 ± 0	3 ± 2.2**	5.8 ± 6**	-
Systolic BP (mm/Hg)	118.38 ± 14.70	122.69 ± 23.17	123.88 ± 13.53	0.633
Diastolic BP (mm/Hg)	72.94 ± 9.98	74.81 ± 12.35	78.63 ± 11.06	0.337
Mean Arterial Pressure MAP (mm/Hg)	88.08 ± 10.52	72.62 ± 33.83	93.71 ± 10.87	0.522

Values are means ± SD unless stated otherwise.

* p < 0.05 significant difference from NS

** *p* <0.001 significant difference from NS
‡ p< 0.05 TC vs. EC

Abbreviations: BP = Blood pressure

3.2 Resting lung function

Groups had comparable baseline pulmonary function and respiratory muscle strength (Table 2).

Table.2 Baseline pulmonary function and respiratory muscle strength in NS, TC and EC.

Variable	NS	EC	TC	*P* value
FVC (L)	4.8 ± 0.9	5.17 ± 1.24	5.03 ± 1.12	0.611
FVC% predicted	102.0 ± 10.49	103.5 ± 13.23	104.94 ± 10.64	0.778
FEV1 (L)	3.9 ± 0.73	4.27 ± 0.86	4.06 ± 0.90	0.489
FEV1 % predicted	95.7 ± 13.2	99.31 ± 9.14	100.94 ± 11.86	0.918
FEV1/FVC (%)	82.95± 5.72	84.19 ± 4.9	80.9 ± 6.33	0.194
IC (L)	3.54 ± 0.66	3.68 ± 0.86	3.94 ± 0.81	0.345
PEF (L/S)	9.13 ± 1.83	7.67 ± 1.23	7.52 ± 1.98	0.113
MEF 75 (L/s)	7.48 ± 1.86	6.81 ± 1.14	6.42 ± 1.75	0.119
MEF 50 (L/s)	4.73 ± 1.35	5.98 ± 0.78	4.33 ± 1.52	0.142
MEF 25 (L/s)	2.09 ± 0.78	2.3 ± 0.69	2.05 ± 0.74	0.838
MVV (L/min)	157.80 ± 37.44	148.04 ± 34.88	146.91 ± 29.09	0.503
MIP (cmH_2O)	107.32 ± 18.58	108.96 ± 20.65	100.43 ± 25.12	0.539
MEP (cmH_2O)	109.26 ± 22.44	115.18 ± 21.57	106.42 ± 30.17	0.528

Values are means ± SD unless stated otherwise.

* p < 0.05

** *p* <0.001

‡ p< 0.05 TC vs. EC

Abbreviations: FEV_1 = Forced expiratory volume in 1 second; FEV_1/FVC = Ratio of forced expiratory volume in the first 1 second to the forced vital capacity; FVC = Forced vital capacity; IC = Inspiratory

capacity; PEF = Peak expiratory flow; MVV = Maximal voluntary ventilation; MIP = Maximal inspiratory pressure; MEP = Maximal expiratory pressure

3.3 Peak cardiorespiratory responses

Table.3 Cardiorespiratory data at peak exercise based on maximal cardiopulmonary exercise test in NS, TC and EC.

Variable	NS	EC	TC	*P* value
Work rate (W)	227.50 ± 43.74 †*	183.75 ± 30.30 †	185.0 ± 34.64 *	0.003
$\dot{V}O_2$ (L/min)	2.92 ± 0.55	2.51 ± 0.47	2.57 ± 0.56	0.919
$\dot{V}O_2$ (ml/kg/min)	42.07 ± 5.78	34.16 ± 5.57	35.73 ± 4.19	0.094
$\dot{V}O_2$ (ml/kg/min-%predicted)	105.36 ± 6.81 †*	97.78 ± 8.50	95.41 ± 6.97 *	0.001
Heart rate (beats/min)	183.78 ± 12.0	180.59 ± 10.73	176.01 ± 12.82	0.270
$\dot{V}O_2$/HR, O_2 pulse	15.78 ± 3.07	13.54 ± 2.71	14.32 ± 3.51	0.280
VCO_2 (L/min)	331.84 ± 60.94	286.02 ± 58.79	284.53 ± 62.98	0.098
$\dot{V}_E$ (L/min)	118.73 ± 29.02 †*	98.48 ± 24.99†	98.19 ± 22.99 *	0.064
V_T (L)	2.55 ± 0.56	2.58 ± 0.63	2.43 ± 0.47	0.729
F_b (breaths/min)	48.19 ± 8.77 †	39.94 ± 7.81 †	42.04 ± 7.76	0.038
$\dot{V}_E/\dot{V}CO_2$	35.71 ± 5.73	34.25 ± 7.81	34.86 ± 5.59	0.813
$P_{ET}CO_2$ (mmHg)	4.52 ± 0.66	4.68 ± 0.56	4.6 ± 0.66	0.703
IC (L)	3.41 ± 0.68	3.64 ± 0.90	3.42 ± 0.57	0.518
BLa (mmol/L)	12.94 ± 2.62	11.73 ± 2.49	12.53 ± 3.84	0.689
Dyspnoea (Borg scale)	7.88 ± 2.25	7.63 ± 1.96	7.59 ± 3.84	0.997
Leg discomfort (Borg scale)	8.63 ± 1.82	8.88 ± 1.45	8.75 ± 1.53	0.611

Values are means ± SD unless stated otherwise.

* $p < 0.05$ NS vs. TC
† $p < 0.05$ NS vs. EC
‡ $p < 0.05$ TC vs. EC

Abbreviations: BLa = blood lactate; F_b = breathing frequency; IC = inspiratory capacity; $P_{ET}CO_2$ = partial pressure of end-tidal carbon dioxide; $\dot{V}_E$ = minute ventilation; $\dot{V}_E/\dot{V}CO2$ = ventilatory equivalent for carbon dioxide; $\dot{V}O2/HR$ or O_2 pulse = oxygen uptake/ heart rate; $\dot{V}O_2$ ml/kg/min- %predicted, $\dot{V}O_2$ ml/kg/min as a % of estimated vo2max; V_T = tidal volume.

There was a significant difference in WR as determined by the one-way ANOVA ($F(2,45) = 7.385$, $p = 0.002$). WR ranged from 180-340 W, 140-260 W, 140W-240W in NS, TC and EC respectively. TC and EC groups reached a lower peak WR compared to NS (185.0 ± 34.64, 183.75 ± 30.30 vs. 227.50 ± 43.74 W; $p = 0.006$ and $p = 0.004$, respectively) (Table 3).

There was a significant difference in F_b as determined by the one-way ANOVA ($F(2,45) = 3.513$, $p = 0.038$). F_b ranged from (32.26-63.68 breaths/min, 33.69-60.74 breaths/min, and 32.28-61.67 breaths/min) in NS, TC and EC respectively. F_b was lower in EC vs NS (39.94 ± 7.81 breaths/min, vs. 47.50 ± 9.49 breaths/min, respectively $p = 0.037$). However, no difference in F_b was found between TC (41.88 ± 7.72, $p = 0.151$ breaths/min) and NS (Table 3).

No significant differences in HR, $\dot{V}O_2/HR$, $\dot{V}_E$, absolute or relative $\dot{V}O_2$, $\dot{V}CO_2$, V_T, $\dot{V}_E/\dot{V}CO_2$, $P_{ET}CO_2$, IC, BLa, dyspnoea or leg discomfort were found between the groups at peak exercise (Table 3).

3.4 Cardiorespiratory responses during exercise

There were no significant differences in $\dot{V}O_2$, HR and O_2 pulse between TC, EC, and NS during the CPET (Fig.1, 2 and 3). At 140W there were no significant differences determined by the ANOVA ($F(2,45) = 0.761$, $p = 0.473$; $F(2,45) = 1.316$, $p = 0.278$); ($F(2,45) = 0.155$, $p = 0.857$) in $\dot{V}O_2$, HR and O_2 pulse respectively between TC, EC, and NS.

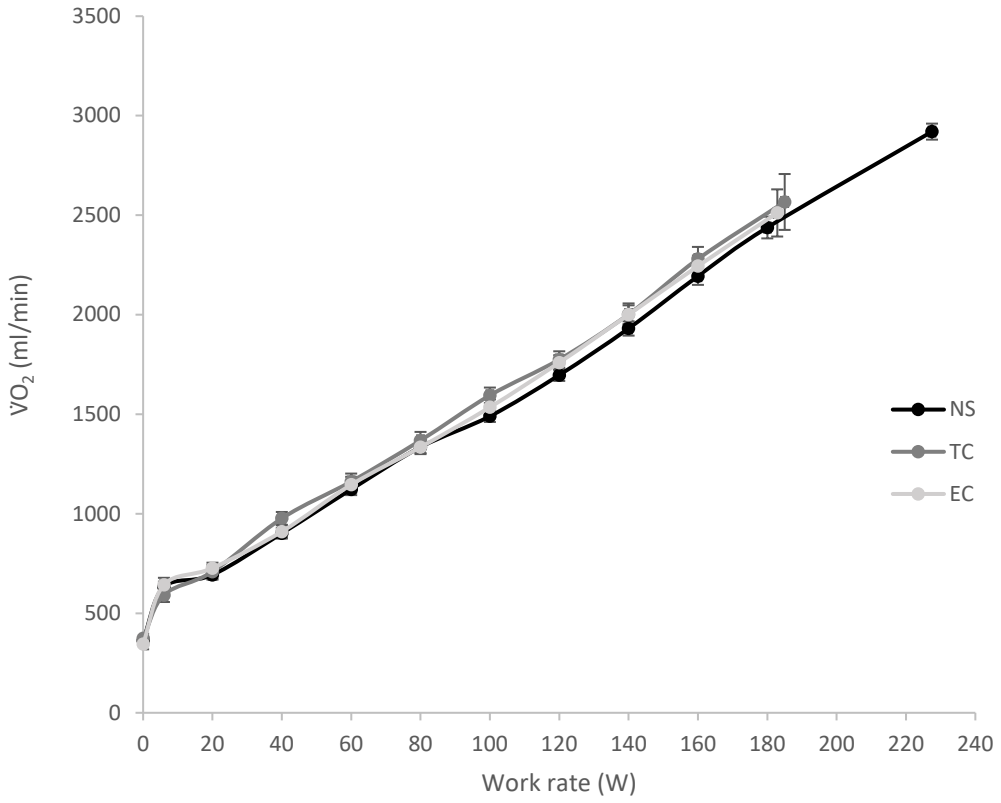

Figure 1. $V\dot{O}_2$ (ml/min) shown relative to work rate (W) during incremental CPET in NS, TC and EC.

* $p < 0.05$ NS vs. TC
† $p < 0.05$ NS vs. EC
‡ $p < 0.05$ TC vs. EC

Values are means ± standard error.

Abbreviations: $V\dot{O}_2$ = oxygen consumption

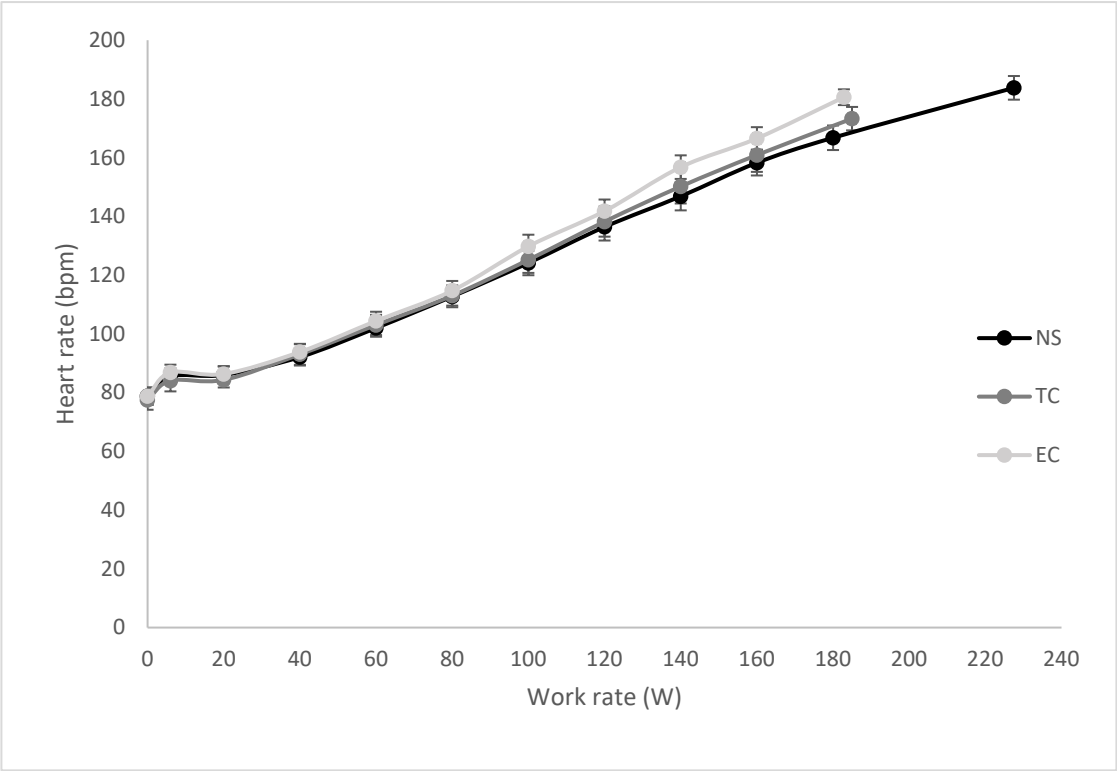

Figure 2. Heart rate (bpm) shown relative to work rate (W) during incremental CPET in NS, TC and EC.

* $p < 0.05$ NS vs. TC
† $p < 0.05$ NS vs. EC
‡ $p < 0.05$ TC vs. EC

Values are means ± standard error.

Abbreviations: bpm = beats per minute

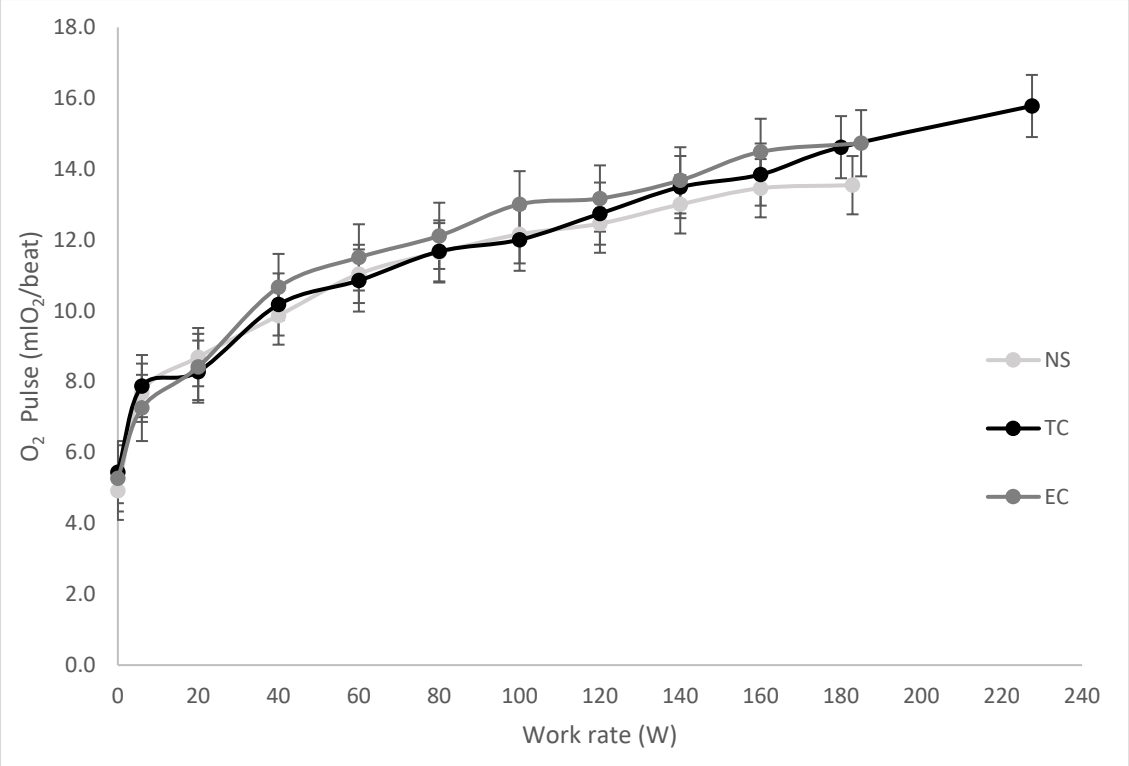

Figure 3. O_2 pulse shown relative to work rate (W) during incremental CPET in NS, TC and EC.

* p < 0.05 NS vs. TC
† p < 0.05 NS vs. EC
‡ p< 0.05 TC vs. EC

Values are means ± standard error.

Abbreviations: V̇O2/HR or O_2 pulse = oxygen uptake/ heart rate

3.5 Ventilatory exercise responses

Throughout exercise, $\dot{V}_E$ (L/min) was higher in TC compared to NS during iso-submaximal workloads 80-160 W and higher in EC compared to NS at submaximal workloads 100-140 W (Fig.4). There was a significant difference in $\dot{V}_E$ as determined by the one-way ANOVA at 140W ($F(2,45) = 5.542$, $p = 0.007$). At 140w $\dot{V}_E$ was higher in TC and EC compared to NS (62.16 ± 9.48 L/min, $p = 0.018$; 64.43 ± 12.34 L/min, $p = 0.011$ vs. 53.52 ± 6.88 L/min, respectively).

Throughout exercise, ratings of dyspnoea were higher in TC compared to NS at iso-submaximal workloads 60-160 W and higher in EC compared to NS at iso-

submaximal workloads 80-140 W (Fig. 8). There was a significant difference in dyspnoea as determined by the ANOVA at 140W ($F(2,45) = 6.756$, $p = 0.003$. At 140 W dyspnoea was higher in TC and EC compared to NS (5 ± 2 Borg units, $p = 0.033$; 6 ± 2 Borg units, $p < .001$ vs. 3 ± 2 Borg units, respectively).

Throughout exercise, $P_{ET}CO_2$ was lower in TC compared to NS at iso-submaximal workloads 40-140 W (Fig. 6). There was a significant difference in $P_{ET}CO_2$ as determined by the ANOVA at 140W ($F(2,45) = 3.437$, $p = 0.041$. At 140W $P_{ET}CO_2$, was lower in TC compared to NS (5.01 ± 0.73 mmHg vs. 5.52 ± 0.45 mmHg, $p = 0.033$, respectively). No significant difference was found between EC and NS, $p = 0.498$.

Throughout exercise, $\dot{V}_E/\dot{V}CO_2$ was higher in TC compared to NS at iso-submaximal workloads 80-140 W (Fig.7). There was a significant difference in $\dot{V}_E/\dot{V}CO_2$ as determined by the ANOVA at 140W ($F(2,45) = 3.322$, $p = 0.045$. At 140W $\dot{V}_E/\dot{V}CO_2$ was higher in TC compared to NS (31.58 ± 5.75, vs. 28.40 ± 2.33, $p = 0.036$, respectively). No significant difference was found between EC and NS, $p = 0.548$.

3.6. Anaerobic threshold

There was a significant difference in the anaerobic threshold (AT) at absolute $\dot{V}O_2$ as determined by the one-way ANOVA ($F(2,45) = 7.084$, $p = 0.002$). The AT was reached at a lower absolute $\dot{V}O_2$ in TC and EC compared to NS (1440.63 ± 377.9 L/min, $p = 0.005$; 1467.81 ± 416.28 L/min, $p = 0.008$ vs. 1908.94 ± 390.12 L/min, respectively).

There was a significant difference in AT reached at WR as determined by the one-way ANOVA ($F(2,45) = 11.045$, $p < .001$). The AT was reached at a lower WR in in TC and EC compared to NS (81.25 ± 35.38 W, $p < .001$; 81.25 ± 31.4 W, $p < .001$ vs. 131.25 ± 37.22 W, respectively).

There was a significant difference in AT % $\dot{V}O_{2\,max}$ as determined by the one-way ANOVA ($F(2,45) = 6.678$, $p = 0.003$). The AT (lactate threshold) occurred at a lower % $\dot{V}O_{2\,max}$ in TC and EC compared to NS (55.9 ± 8.8 L/min, $p = 0.010$; 57.8 ± 8.2

L/min, p = 0.009 vs. 65.4 ± 6.1 L/min, respectively). There was a significant difference AT % WR as determined by the one-way ANOVA (F(2,45) = 11.045, p <.001). The anaerobic threshold occurred at a lower WR in TC and EC compared to NS (43.1 ± 14.8 W, p = 0.022; 44.9 ± 14 W, p = 0.013 vs. 57.4 ± 10.6 W, respectively).

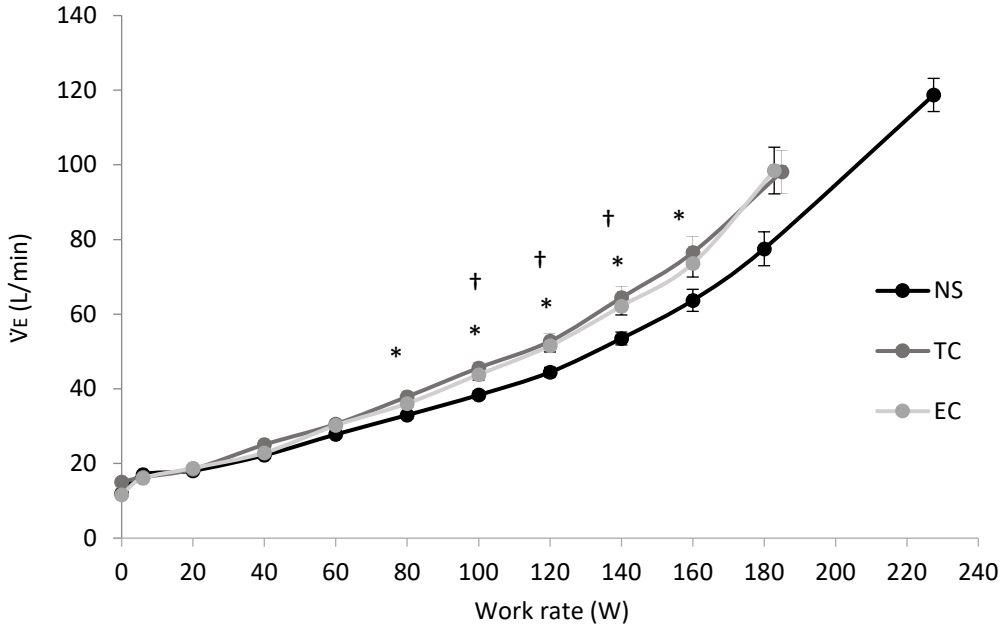

Figure 4. Ventilation is shown relative to work rate during incremental CPET in NS, TC and EC.

* p < 0.05 NS vs. TC
† p < 0.05 NS vs. EC
‡ p< 0.05 TC vs. EC

Values are means ± standard error.

Abbreviations: $\dot{V}_E$ = Minute ventilation

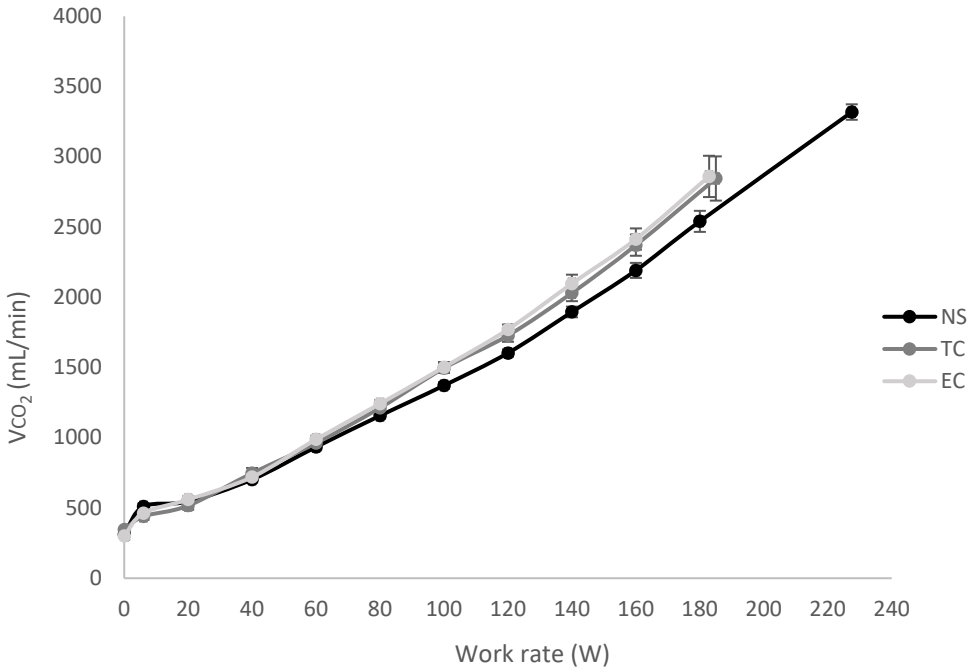

Figure 5. VCO_2 shown relative to work rate (W) during incremental CPET in NS, TC and EC.

* $p < 0.05$ NS vs. TC
† $p < 0.05$ NS vs. EC
‡ $p < 0.05$ TC vs. EC

Values are means ± standard error.

Abbreviations: VCO_2 = Carbon dioxide production

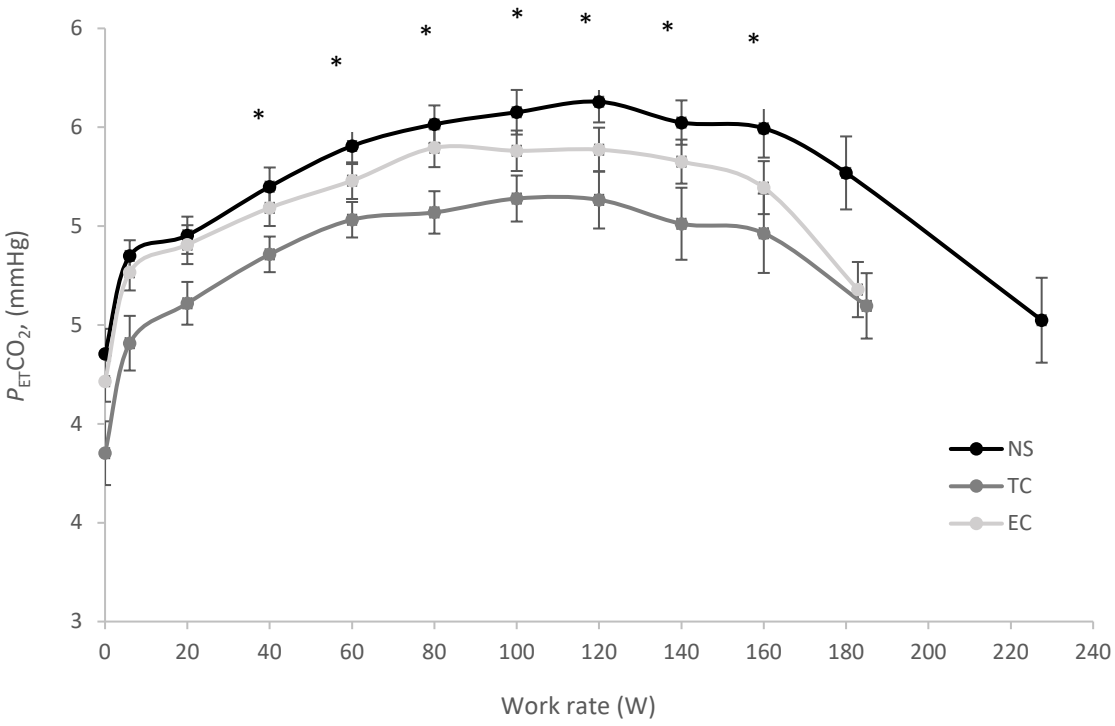

Figure 6. Partial pressure of end-tidal carbon dioxide is shown relative to work rate during incremental CPET in NS, TC and EC.

* $p < 0.05$ NS vs. TC
† $p < 0.05$ NS vs. EC
‡ $p < 0.05$ TC vs. EC

Values are means ± standard error.

Abbreviations: $PetCO_2$ = Partial pressure of end-tidal carbon dioxide

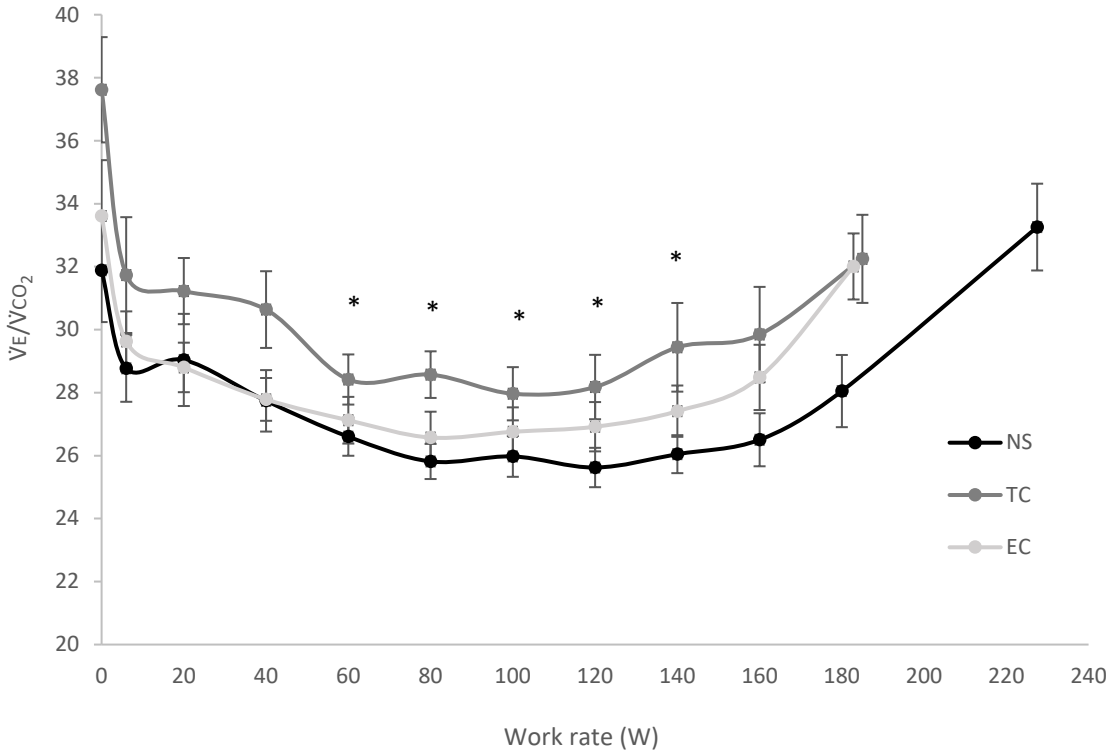

Figure 7. Ventilatory equivalent for carbon dioxide is shown relative to work rate during incremental CPET in NS, TC and EC.

* $p < 0.05$ NS vs. TC
† $p < 0.05$ NS vs. EC
‡ $p < 0.05$ TC vs. EC

Values are means ± standard error.

Abbreviations: $\dot{V}_E / \dot{V}CO_2$ = Ventilatory equivalent for carbon dioxide

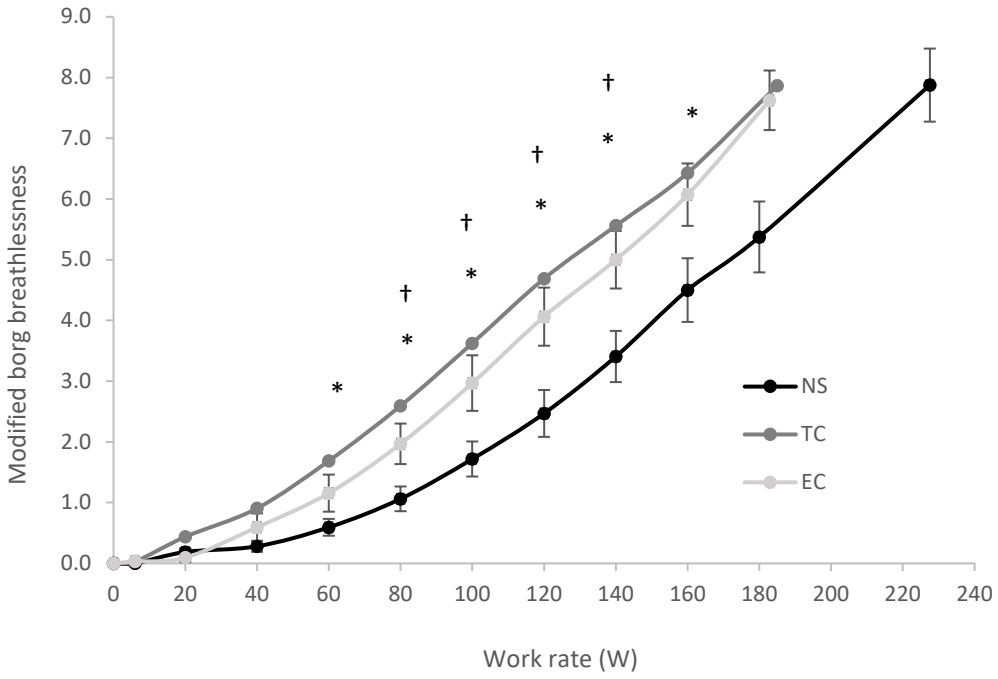

Figure 8. Breathlessness is shown relative to work rate during incremental CPET in NS, TC and EC.

* $p < 0.05$ NS vs. TC
† $p < 0.05$ NS vs. EC
‡ $p < 0.05$ TC vs. EC

Values are means ± standard error.

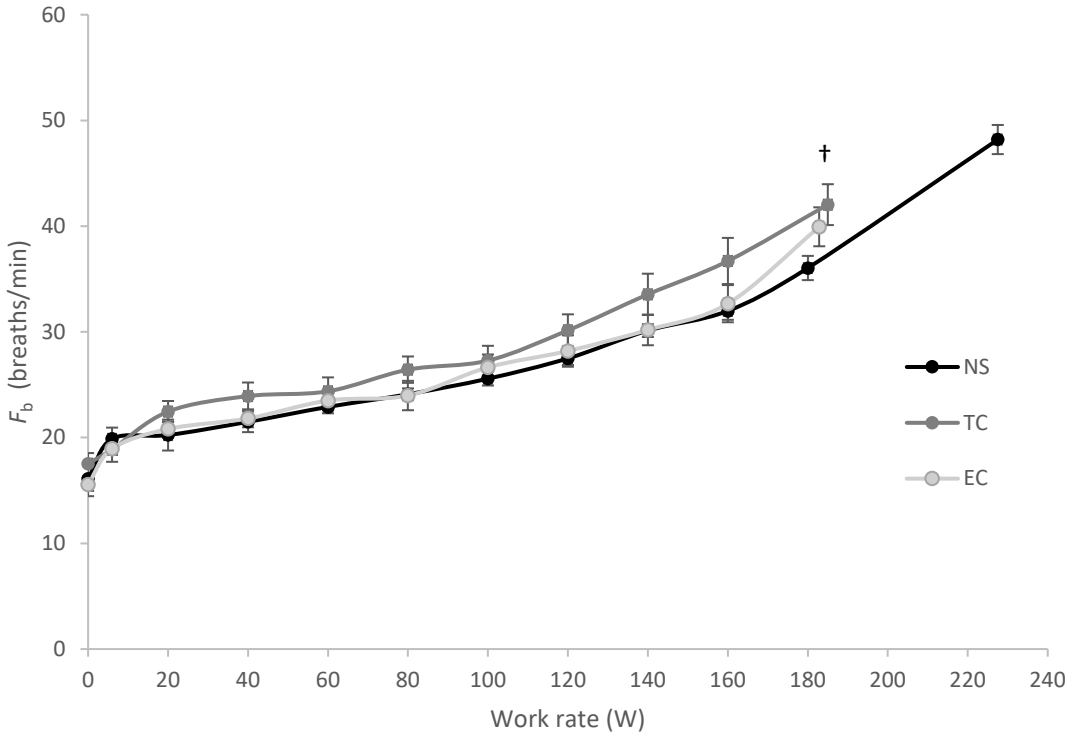

Figure 9. Breathing frequency is shown relative to work rate during incremental CPET in NS, TC and EC.

* p < 0.05 NS vs. TC
† p < 0.05 NS vs. EC
‡ p< 0.05 TC vs. EC

Values are means ± standard error.

Abbreviations: F_b = Breathing frequency.

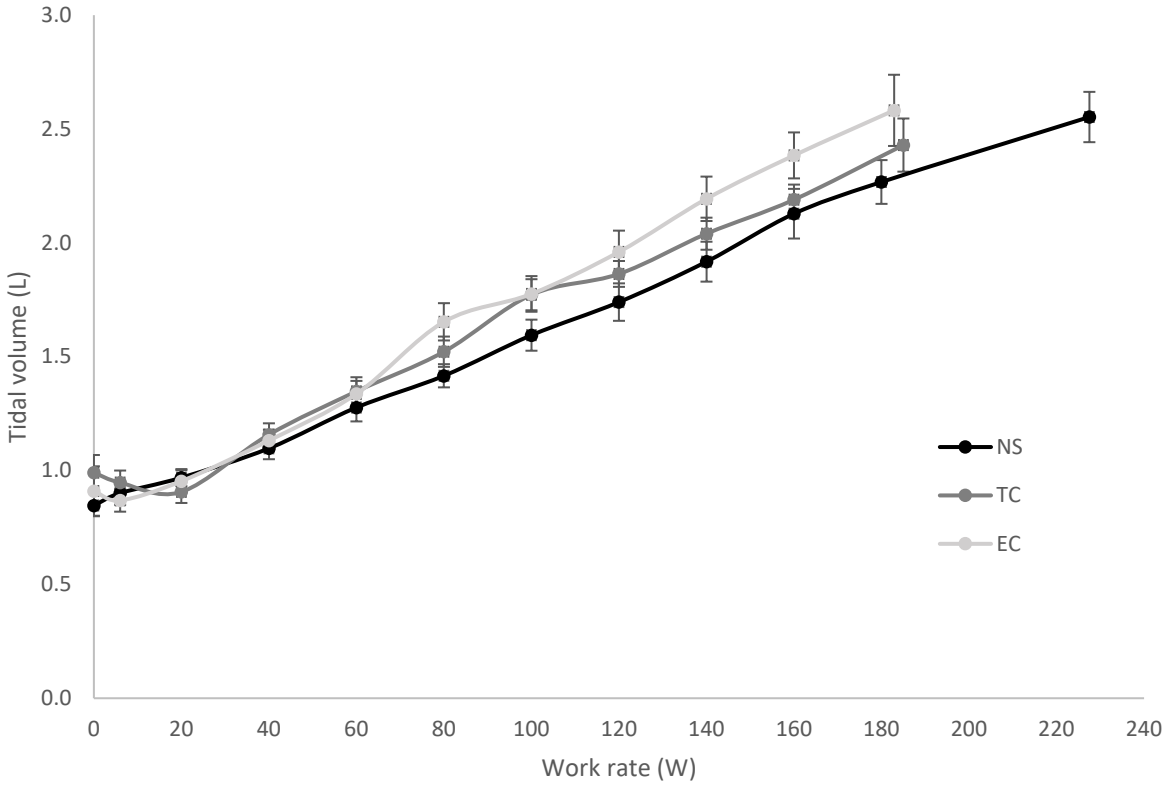

Figure 10. Tidal volume is shown relative to work rate during incremental CPET in NS, TC and EC.

* p < 0.05 NS vs. TC
† p < 0.05 NS vs. EC
‡ p< 0.05 TC vs. EC

Values are means ± standard error.

3.7 Ratings of perceived leg fatigue and blood lactate

BLa was higher in TC compared to NS at iso-submaximal workloads 120-160 W (Fig.11). There was a significant difference in BLa as determined by the one-way ANOVA ($F(2,45) = 6.567$, $p = 0.003$). At 140W BLa was higher in TC compared to NS (6.54 ± 2.42 mmol/L, $p = 0.030$ vs. 4.29 ± 2.45 mmol/L). No significant difference was found between EC and NS, $p = 0.070$).

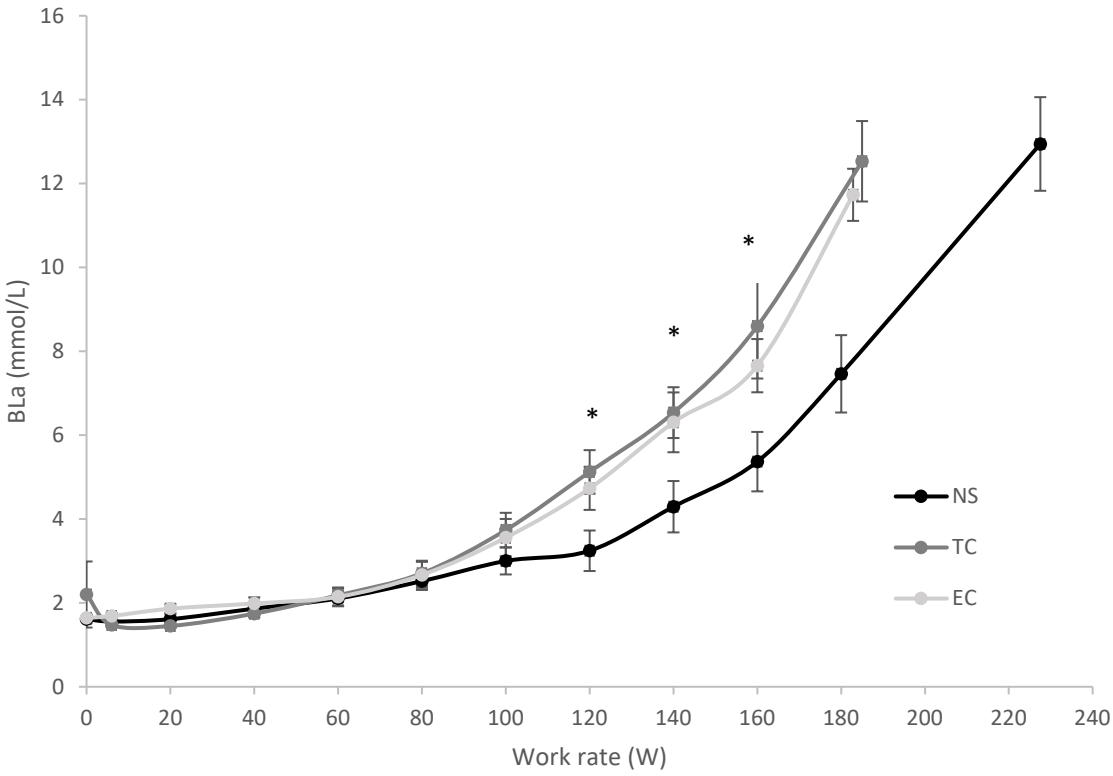

Figure 11. Blood lactate is shown relative to work rate (W)

* p < 0.05 NS vs. TC
† p < 0.05 NS vs. EC
‡ p< 0.05 TC vs. EC

Values are means ± standard error.

Abbreviations: BLa = Blood lactate, mmol/L = Millilitres of mercury

There was a significant difference in LF as determined by the one-way ANOVA ($F(2,45) = 8.607$, $p < .001$). Ratings of LF were higher in TC compared to NS at iso-submaximal workloads 40-160 W and higher in EC compared to NS at iso-submaximal workloads 60-140 W (Fig.12). At 140 W LF was higher in TC and EC compared to NS (7 ± 2 Borg units, $p = 0.014$; 7 ± 2 Borg units, $p = 0.048$ vs. 5 ± 3 Borg units, respectively).

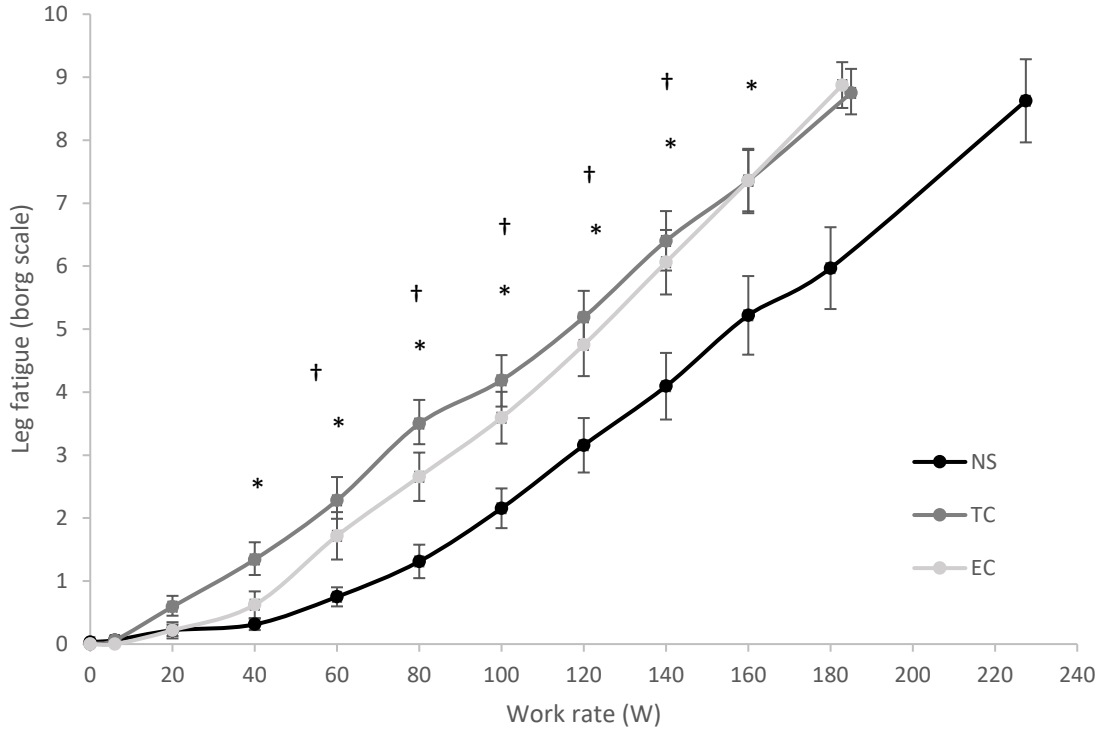

Figure 12. Leg fatigue is shown relative to work rate (W)

* p < 0.05 NS vs. TC
† p < 0.05 NS vs. EC
‡ p< 0.05 TC vs. EC

Values are means ± standard error

3.8 FMD

There was a significant difference in FMD% as determined by the one-way ANOVA ($F(2,39) = 4.993$, $p = 0.012$). FMD% was lower in TC and EC compared to NS (5.26 ± 3.09%, $p = 0.040$; 4.85 ± 2.55%, $p = 0.017$ vs. 8.14 ± 3.46%, respectively) (figure.13).

There was no significant difference as determined by the one-way ANOVA ($F(2,39) = 0.610$, $p = 0.548$; $F(2,39) = 0.231$, $p = 0.795$; $F(2,39) = 0.908$, $p = 0.412$; $F(2,39) = 0.981$, $p = 0.384$), in baseline diameter, peak diameter, shear rate Area to max or time to peak flow, respectively (figure.13).

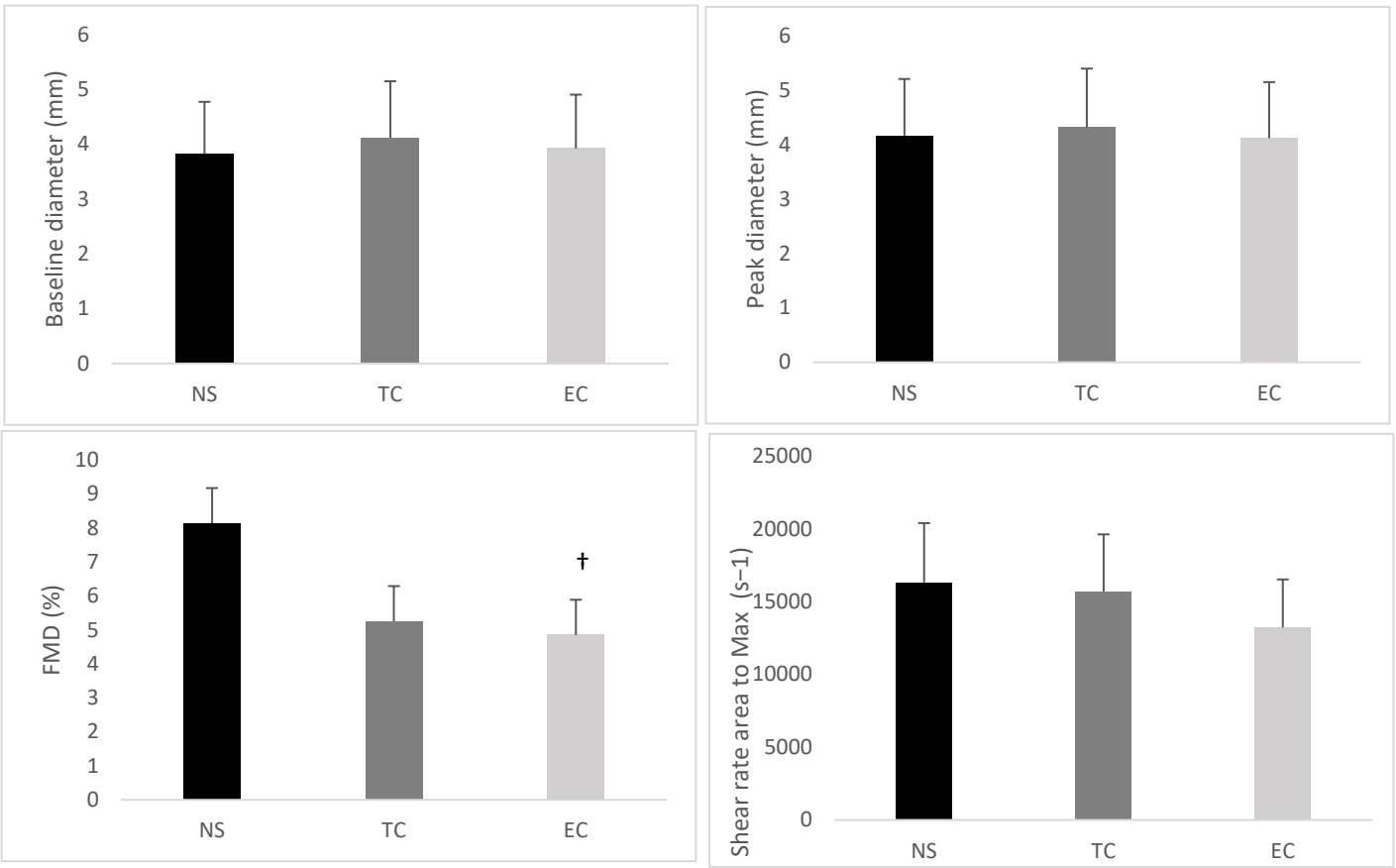

Figure 13. Flow mediated dilation, baseline diameter, peak diameter and shear rate to Area max in TC, EC and NS

* $p < 0.05$ NS vs. T
† $p < 0.05$ NS vs. EC
‡ $p < 0.05$ TC vs. EC

Values are means ± standard error.

Abbreviations: FMD = Flow mediated dilation, mm = millimetres, s = seconds

4.Discussion

The novel finding of this study was that in a cohort of young adults aged 18-30 without airflow limitation, chronic EC users showed impaired resting vascular function (lower FMD), higher ventilatory inefficiency (higher $\dot{V}_E/\dot{V}CO_2$), higher $\dot{V}_E$, BLa, and symptoms of dyspnoea and leg fatigue to a similar extent as tobacco cigarettes smokers during submaximal exercise workloads compared to NS. These findings challenge the idea of using EC use is a 'healthier alternative' to tobacco cigarettes smoking.

4.1 Respiratory function

Based on the inclusion criteria there were no differences in lung function between the testing groups (Table 2). However, previous studies report that both EC use and TC smoking cause a decline in lung function. Smoking TC is the main cause of a decline in FEV_1 (Fletcher et al.,1975; Lange et al.,1989). FVC has also been reported to decline by 14.17 mL per year in TC smokers compared to NS (Tian et al., 2023). This lung function decline in TC smokers has been associated with the elevation of the number of neutrophils, mast cells and eosinophils in TC smoker's lungs. Additionally, TC smoking decreases the number of airway dendritic cells and impairs the roles of macrophages and neutrophils (Mehta et al., 2008; Sopori et al., 2002). Ishida et al., (2009) study highlighted that TC smoking impairs the function of macrophages by increasing ROS production (superoxide and hydrogen peroxide). They reported that oxidative stress results in the inhibition of B lymphocyte proliferation in response to lipopolysaccharide stimulation and this inhibition was reversed after the addition of antioxidants including superoxide dismutase and catalase. This suggests that oxidative stress caused by TC smoking is a main contributor for the impairment of macrophage-mediated immune responses (Ishida et al., 2009). Previous literature has also reported a lower MVV in TC smokers compared to NS. Padmavathy, (2008) suggested that this could potentially be due to a decreased strength of the respiratory muscles. Birajdar et al., (2016) also suggested that a reduction in MVV could also be related to smoking-related oxidative

stress caused by direct damage by radical species and smoking-induced inflammatory response and because MVV is indicative of neuromuscular co-ordination and elastic and flow resistance of the respiratory system. This decreased strength in respiratory muscles coincides with Melliti, et al., (2021) study which although no differences were reported in MEP, their study revealed a decreased MIP in TC compared to NS. Melliti, et al., (2021) associated this decrease in respiratory muscle strength with dynamic hyperinflation in TC which increases the oxygen cost of breathing. Additionally, Meo et al., (2019) reported a decline in lung function parameters in EC users. This included FEV_1, FEV_1/FVC ratio, $FEF_{50\%}$, $FEF_{75\%}$, $FEF_{25\%–75\%}$, and $FEF_{75\%–85\%}$. This could display peripheral obstructive airway impairment in EC users (Meo et al., 2019). In contrast, Vardavas et al., (2012) study found no differences in FVC, FEV_1, and FEV_1/FVC ratio from using EC for 5 minutes. The present study reveals no differences in the lung function parameters; FVC, FEV_1, FEV_1/FVC, IC, MVV or respiratory muscle strength (MIP or MEP) in EC and TC in comparison to NS. However, this is somewhat expected as we included young adults with normal spirometry.

4.2 The effect of tobacco cigarette smoking and e-cigarette use on the cardiovascular responses to exercise

The present study found that FMD% was lower to a similar extent in both EC users and TC smokers compared to NS (Fig. 13). This aligns with a previous study that concluded that both chronic EC and tobacco smoking decrease FMD and lead to decreased vascular endothelial growth factor (VEGF) induced NO secretion and a decreased bioavailability of NO (Mohammadi et al., 2022). Endothelial dysfunction has been reported to be an early and sensitive indicator of oxidative damage in both EC users and TC smokers (Haptonstall et al., 2020). Elevated inflammatory marker levels in EC users and TC may have contributed to the lower FMD found in EC users and TC smokers compared to NS. This includes raised levels of plasma circulating intercellular adhesion molecule (ICAM-1) concentration in EC users and TC smokers (Bermudez et al., 2002; Mohammadi et al., 2022), which is associated with endothelial dysfunction (Szmitko et al., 2003). Furthermore, studies highlighted that TC smokers (Lavi et al., 2007) and EC users (Mohammadi et al., 2022) have

significantly higher levels of MPO than NS. MPO leads to impaired endothelial function by inhibiting NO availability and enhancing oxidative stress (Hartman and Ford, 2018). H_2O_2 has been reported to be elevated in EC and TC, another reactive oxygen species (ROS), which mediates endothelial cell function through the Fas cell signalling pathway (Nowak et al.,1996; Suhara et al., 1998). This reveals that TC smoking and EC use leads to elevated endothelial oxidative stress which results in endothelial dysfunction. it has been suggested that increased oxidation stress of lipoprotein lipase (LDL) due to an increase in reactive oxygen species (ROS) (including the free radicals (superoxide anion and hydroxyl radicals) found in the particulates of TC smoke (Chatterjee, et al., 2007; Ceriello., 2008) inhibit eNOS and the production of NO (Barua et al., 2001; Barua et al., 2003). However, the role of eNOS inhibition in NO production in EC users has not yet been confirmed (Mohammadi et al., 2022).

4.3 Impact of tobacco smoking and e-cigarette use on $\dot{V}O_{2\ max}$ and exercise capacity

Endothelial dysfunction has been associated with impaired exercise capacity (Hagg, et al., 2005). Therefore, the impaired endothelial function found in both EC users and TC smokers in this study could contribute to exercise intolerance in both groups. This coincides with a previous study which reported a decrease in exercise capacity by 30.8% in middle-aged smokers (Sadaka et al. 2021) and over 25% in elderly TC smokers compared to NS (Elbehairy et al., 2016). The mechanisms behind this impairment are unclear in EC users. However, in TC smokers, this decline in exercise capacity is postulated to be caused by an increase in carbon monoxide (CO) in the blood (Ekblom and Huot, 1972), which can decrease the availability of oxygen to the working muscles because of compromised redistribution of blood flow during exercise (Hirsch et al., 1985). Another theorised mechanism behind reduced exercise capacity (a lower $\dot{V}O_2max$) includes increased anaerobic metabolism due to increased carboxyhaemoglobin (COHb) levels (Araújo et al., 2004). The reduced oxygen supply and increased COHb levels to the tissues increase endothelial permeability (Bullen, 2008). This study found a decrease in the anaerobic threshold in TC and EC compared to NS with a 21.8% lower work rate in EC and at a 24.9% lower work rate in TC compared to NS and at a 11.6% lower % $\dot{V}O_2max$ in EC and at

a 14.5% lower % $\dot{V}O_2$max in TC. This metabolic acidosis alongside damage to the intima of the arterial wall related to CO exposure, results in sub-endothelial oedema due to early atherosclerosis including the build up of fat substances in the arterial walls (Kjeldsen et al., 1969). CO has been reported to decrease the bioavailability of NO, by inhibiting eNOS activity (Abu-Soud et al., 1998). This Inhibition of eNOS has been related to increased vasoconstriction (Pucci et al., 1992). This inhibition of eNOS, alongside impaired vasodilation and oxidative stress, may lead to endothelial dysfunction (Papathanasio et al., 2014). Although no differences were found in $\dot{V}O_2$max between TC smokers, EC users and NS controls, this could be a result of small sample size. The shift in anaerobic metabolism and reduced vascular function indicate impaired oxygen delivery and reduced exercise capacity in TC and EC groups.

There are limited studies reporting the impact of e-cigarette use on $\dot{V}O_2$max. One unpublished master's thesis which had a small sample size of only 17 subjects; n=10 NS and n=7 EC users, highlighted that 6 months of vaping showed no differences in $\dot{V}O_2$max (Robinson, 2020), whereas the present study included EC users that had used e-cigarettes for at least 18 months. This may be relevant as $\dot{V}O_2$max in tobacco smokers has been found to be dose dependent. This dose-dependent impact on $\dot{V}O_2$max is highlighted by Suminski et al., (2009) study which reported a lower $\dot{V}O_2$max in heavy smokers compared to light smokers (groups depended on the number of packs smoked per year).

Furthermore, this study found no differences in in EC and TC in HR and HR_{max} compared to NS. This could be related to the young age of the participants in this study. The inability of tobacco smokers to reach HR_{max} has been associated with impaired exercise capacity (McDonough and Moffatt, 1999). This study opposes studies that found a decrease in HR_{max} in smokers compared to non-smokers (Sandvik et al., 1995; Savonen et al., 2006). A compromised b-adrenergic effect (Toda and Okamura., 2003) and endothelial dysfunction have been associated with decreases in HR during exercise as an adaptation to long-term smoking (Lauer et al., 1997; Bernaards et al., 2003; Toda and Okamura., 2003). This down-regulation of β-adrenergic receptors caused by a decreased lymphocyte density and an impaired catecholamine response, is evidenced by decreased ligand binding and

decreased cyclic AMP formation in response to isoproterenol (Laustiola et al., 1988). Additionally, the lower plasma cyclic AMP levels and reduced free fatty acids during exercise in smokers suggest β-receptor downregulation in adipose tissue (Laustiola et al., 1988). These physiological adaptations collectively result in a blunted HR increase (Papathanasiou et al., 2013). HR in TC smokers is known to increase at a slower rate than non-smokers during exercise, leading to the inability of reaching an as high HR at peak exercise (Papathanasiou et al., 2013). These studies included smokers with a low physical activity and an impaired cardiorespiratory fitness (Sandvik et al., 1995; Savonen et al., 2006; Lauer et al., 1996), whereas this study may have found no differences in HR and HR_{max} because this study included EC and TC who were 'active' participants as shown with ~100% predicted VO_2max in all testing groups (Table 3). In contrasting studies, it has been postulated that this reduced ability of TC to achieve HR_{max} is related to chronotropic incompetence (Lauer, 2004) and the inability of HR to increase during exercise to reach metabolic demands (Zweerink et al., 2018). This can result in impaired exercise capacity due to tobacco cigarette smoker's inability to execute a sufficient exercise response (McDonough and Moffatt, 1999). However, as a result of the young age of participants in this study, it is not surprising that we did not find no differences in HR between EC, TC and NS throughout submaximal or at peak exercise.

No significant differences in HR were found in EC. Previous literature on HR response during exercise is very limited and more ambiguous. However, in line with this study's findings, Polosa et al., (2017) found no changes in HR between EC and NS during exercise. In contrast, a meta-analysis comprising of 11 studies and 283 participants determined that e-cigarette use does increase HR at rest (Skotsimara et al., 2018) similar to TC smoking but potentially less apparent (Yan and D'Ruiz., 2015). This increase in HR could be associated with nicotine by sympathetic neural mechanisms as well as the systematic release of catecholamines (Benowitz and Fraiman, 2017).

It is postulated that HR and O_2 pulse ($\dot{V}O_2$/HR) are related to cardiac function (Come et al., 2012). Therefore, no differences in O_2 pulse found in EC and TC could be linked to no differences in HR. In contrast to this study's findings, Kobayashi et al., (2004) study found a decrease in O_2 pulse throughout exercise in TC compared to

NS. In addition, a lower O_2 pulse at peak exercise has been attributed to impaired vasodilation to the working muscles (Hirsch et al., 1985).

4.4 Ventilatory and dyspnoea responses during exercise in tobacco cigarette smoking and e-cigarette users

During submaximal exercise (140 W) $\dot{V}_E$ was 16.23% higher in EC and 20.47% higher in TC than NS. This coincides with Sadaka et al., (2021) study which also found an increase in submaximal $\dot{V}_E$ in TC compared to NS. There are no previous studies that have investigated ventilatory responses during exercise in EC. This ventilatory inefficiency could be associated with an increase in physiological dead space and a lower partial pressure of carbon dioxide ($PaCO_2$) (Naeije., 2014). However, this study did not test for these mechanisms. This aligns with previous studies that found elevated $\dot{V}_E/\dot{V}CO_2$ in TC during submaximal and at peak exercise (Sadaka et al., 2021; Melliti et al., 2021; Barbosa et al., 2017). Contrastingly, one study revealed no differences in $\dot{V}_E/\dot{V}CO_2$ in tobacco smokers without airflow limitation compared to healthy non-smoker controls (Elbehairy et al., 2016).

While no previous study has examined $\dot{V}_E$ and $\dot{V}_E/\dot{V}CO_2$ responses in EC users, the increased $\dot{V}_E$ and $\dot{V}_E/\dot{V}CO_2$ in TC and increase in $\dot{V}_E$ in EC users could indicate ventilatory inefficiency alongside the increased physiological dead space (Sadaka et al., 2021; Barbosa et al., 2017). Moreover, mechanisms that could be partially responsible for the increase in $\dot{V}_E/\dot{V}CO_2$ in TC smokers include bronchoconstriction, early-onset of emphysema and a decrease in diffusion capacity (Barberá et al., 2001; Gordon et al., 2011). Moreover, an increased ventilatory drive because of an increased sympathetic activity caused by nicotine and earlier metabolic acidosis has been reported to increase $\dot{V}_E$ in TC (Parshall et al., 2012), which is apparent in the present study due to the lower anaerobic threshold in TC. Furthermore, this decrease in ventilatory inefficiency (higher $\dot{V}_E/\dot{V}CO_2$) could be associated with the lower $PetCO_2$ during submaximal exercise in TC compared to NS. This is because $\dot{V}_E/\dot{V}CO_2$ has been reported to correlate with $PetCO_2$ (Yuan et al., 2020). However, no difference in $PetCO_2$ was found during submaximal exercise in EC, which could suggest that TC impairs ventilatory efficiency to a greater extent compared to EC.

Overall, in TC, a lower submaximal $PetCO_2$ and higher $\dot{V}_E/\dot{V}CO_2$ could show changes in pulmonary gas exchange during exercise prior to apparent airflow limitation (Elbehairy et al., 2016; Clark et al., 2020; Elbehairy et al., 2015).

Rotstein et al., (1991) study reported a decrease in V_T and increased F_b in TC during exercise. They suggested that this could be linked to a decrease in gas exchange efficiency and a decrease in peak $\dot{V}O_2$ (Hueper et al., 2015). This contrasts this study which found no differences in $\dot{V}O_2$max. Furthermore, a higher F_b and lower V_T in TC smokers in this study could be attributed to an increase in physiological dead space (Lifshay et al., 1971). There are no previous reports on the impact of EC users on F_b and V_T. Contrastingly to Rotstein et al., (1991) study, no differences in F_b and V_T were found in EC users and TC smokers during submaximal exercise compared to NS. However, increased $\dot{V}_E$ in this study during submaximal exercise in EC users, similarly to the increase in $\dot{V}_E$ in TC smokers, could be related to increased sympathetic activity due to the nicotine content in EC as well as earlier metabolic acidosis, which is demonstrated by the lower anaerobic threshold in this study in EC users compared to NS (Parshall et al., 2012). Increased ventilatory demand in EC users and TC smokers, in this present study, was linked with increased ratings of dyspnoea in EC users compared to NS by 46.63% at 140 W and by 42.89% at 140 W in TC smokers compared to NS. This study is in accordance with Elbehairy et al., (2016) study which also found increases in ratings of dyspnoea at sub-maximal level in a healthy cohort of smokers without airflow limitation. The present study is the first study that investigates ratings of dyspnoea in EC users during exercise. A mechanistic study may be beneficial to explain possible mechanisms of dyspnoea in EC users, including investigating the dead space-to-tidal volume ratio (VD/VT) and diaphragmatic contractile work to overcome increased airway resistance, higher electrical activation and decreased maximal activation (Elbehairy et al., 2016; Sadaka et al., 2021). However, similarly to Sadaka et al., (2021) findings in TC smokers without airflow limitation, no significant divergences were found in TV or inspiratory capacity, suggesting that mechanical ventilatory limitation was not a predominant mechanism for higher ratings of dyspnoea or exercise intolerance (Sadaka et al., 2021).

The present study found higher ratings of leg fatigue throughout submaximal exercise in both EC users and TC smokers compared to NS. This could be attributed to changes in muscle function and increased motor output (Sadaka et al. 2021). Carbon monoxide and ROS in TC smoke causes a left shift in the oxyhaemoglobin dissociation curve (McDonough and Moffatt., 1999). This, alongside the endothelial dysfunction found in EC users and TC smokers in this study could have impaired mitochondrial oxidative capacity resulting in impaired muscle function and increased metabolic acidosis (de Oca et al., 2008; Petersen, et al., 2007; Kirby et al., 2013; Adami et al., 2017; Johnson, et al., 2010) with increased blood lactate during exercise (Colberg et al., 1995). This is shown by an increased blood lactate in TC smokers and EC users compared to NS. This is translated with higher perceived ratings of leg fatigue by 41% in both EC users and TC smokers at 140 W compared to NS. It has been reported that quadriceps endurance is reduced in tobacco smokers (Sadaka et al., 2021). Smokers experience a shift in muscle fibre composition from slow twitch type I, oxidative fatigue-resistant fibres to fast twitch, glycolytic type II fibres (de Oca et al., 2008; ÖRLANDER et al., 1979). The higher ratings of leg fatigue and higher BLa in EC users and TC smokers in this study and afferent stimuli from peripheral mechanoreceptors could have resulted in increased ventilatory drive as well as the findings of higher $\dot{V}_E$ and increased ratings of perceived dyspnoea during submaximal exercise in this study (Parshall et al., 2012). Furthermore, BLa induced carboxyhaemoglobin alongside tobacco smoke injury to the mitochondrial respiratory chain leads to elevated intracellular oxidants (Cardellach et al., 2003; Smith et al.,1993) This is translated with a lower AT in EC users and TC smokers. This higher blood lactate and lower AT in EC users and TC smokers compared to NS could potentially contribute to the increased sensations of dyspnoea and leg fatigue found in the present study. AT is linked to the level of work that can be performed for a period of time (Wasserman et al., 1984). Therefore, the lower AT in EC users and TC smokers found in this study can be linked to the lower work rate achieved.

Overall, increases in blood lactate and leg fatigue in EC users and TC smokers, alongside afferent stimuli from peripheral mechanoreceptors, can be linked to the increased ventilatory drive, dyspnoea, and ventilation which are also found in EC users and TC smokers in the present study (Parshall et al., 2012). It is likely that EC

users and TC smokers terminated exercise earlier (as seen by the lower work rate reached in both EC users and TC smokers) when symptoms of leg fatigue and dyspnoea became intolerable (Elbehairy et al., 2016) at a lower $\dot{V}O_2$max. We tested active young individuals with high predicted $\dot{V}O_2$max values in all three groups, therefore it can be assumed that muscle deconditioning was not a main contributor for reduced exercise capacity or increased symptoms of leg fatigue and dyspnoea (Sadaka et al., 2021).

5.Limitations

Despite the interesting results, limitations are apparent. This study has a relatively small sample size (n=16), however it is sufficient to determine consistent clinically significant differences linked to exercise intolerance in EC users and TC smokers. This study included young, habitually active adults with average predicted $\dot{V}$ $\dot{V}O_2$max around 100% so the results cannot be generalised to the general population. However, it could be expected that the responses would be worse in sedentary individuals due to muscle deconditioning. In addition, the differences we have seen in young TC smokers and EC users are expected to be augmented in older and inactive population. The average e-cigarettes used per week was calculated, however, there is a vast variety of vape devices with varying amounts of nicotine, therefore, it is difficult to calculate a precise history of EC dose usage. Blood nicotine and cotinine levels were not measured, which would be beneficial to compare nicotine use between the EC and TC groups. Participants did not smoke or consume any other forms of nicotine outside of their group category. However, one participant in the EC group had a short history of tobacco smoking 5 years before switching to e-cigarettes, but this should not have a significant impact on the responses we have seen in EC group. A few participants had undergone exercise testing at different times of day which could have caused diurnal variations in exercise capacity. However, circadian rhythm has shown no impact on exercise responses in active individuals (Faisal et al., 2010). All vascular function for FMD testing was performed by Dr Rehan Junejo, with the majority of testing performed in the morning (between 9am and 11am), however two of the participant's FMD test included in this study was performed at 12:00. This should not have contributed to significant differences in FMD between the groups. All participants completed spirometry and CPET, however

this study performed FMD on 14 participants in each group. Although the power calculation of this study determined that 16 participants are needed to see significant differences in FMD, this study still showed significant differences between the groups. This study did not measure DL_{CO} as we were not able to meet the health and safety regulations at our new facility prior to the start of data collection.

6. Future Research Studies

This study presents novel observational findings and more detailed physiological studies to rule out the mechanisms associated with reduced exercise capacity and augmented symptoms of breathlessness in EC users are needed. A detailed lung function testing including diffusing capacity of the lungs for carbon monoxide (DLco), N_2 washout testing with the investigation of gastric pressure, oesophageal pressure, and diaphragmatic electromyography (EMG_{di}) using oesophageal and gastric balloons with a combined EMG_{di} electrode catheter (Elbehairy et al., 2016; Sadaka et al., 2021) will rule out the impact of EC on lung mechanics and work of breathing and mechanisms of dyspnoea in EC users.

There is limited literature reporting the impact of EC on cardiac function and detailed assessments of the long-term impacts of cardiac geometry and function could be beneficial to add more precise explanations to the impact of EC on O_2 delivery and HR responses during exercise.

This study's interesting findings of higher exercise BLa and early acidosis in EC and TC groups may suggest a greater impact of EC on peripheral muscles before apparent impact on lung function. A detailed assessment of muscle metabolism using magnetic resonance spectroscopy would add significant value to literature.

In addition, the study of former TC smokers who have switched to e-cigarettes could add further details about the implication of EC users in smoking cessation programmes.

7. Conclusion

This study showed that tobacco smoking and electronic cigarette use impair the cardiorespiratory responses (TC and EC achieved a lower peak work rate, and during submaximal exercise, this study found higher ratings of dyspnoea and leg fatigue, higher $\dot{V}_E$, in EC and TC and lower $P_{ET}CO_2$, higher $\dot{V}_E/\dot{V}CO_2$, lower BLa in TC, and EC and TC reached the AT at a lower work rate and $\dot{V}O_2$max compared to NS) during and at peak exercise to a similar extent in a cohort of young smokers with normal spirometry. This study found increased endothelial dysfunction in EC and TC as shown by the lower FMD compared to NS. A lower FMD% has been associated with impaired exercise capacity (Hagg, et al., 2005). Therefore, it is surprising that this study found no differences in $\dot{V}O_2$max however, the lower FMD% could have been one of many underlying causes that lead to the impaired cardiorespiratory responses to exercise found in this study. The present study found a higher $\dot{V}_E$ in EC and TC during submaximal exercise which could be linked to increased ventilatory inefficiency (higher $\dot{V}_E/\dot{V}CO_2$) in EC and TC compared to NS. In addition, increased FMD, higher BLa and lower AT in EC and TC compared to NS could have contributed to the increased sensations of dyspnoea and leg fatigue found in the present study in TC and EC smokers. Due to these findings, it may be dangerous to assume that e-cigarette use is an alternative with less cardiovascular risk to health than tobacco smoking. The safety of using e-cigarettes as a smoking cessation tool should be investigated further.

8.References

Abu-Soud, H.M., Wu, C., Ghosh, D.K. and Stuehr, D.J., 1998 ‘Stopped-flow analysis of CO and NO binding to inducible nitric oxide synthase.’ *Biochemistry*, *37*(11), pp.3777-3786.

Adami, A., Cao, R., Porszasz, J., Casaburi, R. and Rossiter, H.B., 2017 ‘Reproducibility of NIRS assessment of muscle oxidative capacity in smokers with and without COPD.’ *Respiratory physiology & neurobiology*, *235*, pp.18-26.

Adams, M.R., Jessup, W. and Celermajer, D.S., 1997 ‘Cigarette smoking is associated with increased human monocyte adhesion to endothelial cells: reversibility with oral L-arginine but not vitamin C.’ *Journal of the American College of Cardiology*, *29*(3), pp.491-497.

Ambrose, J.A. and Barua, R.S., 2004 ‘The pathophysiology of cigarette smoking and cardiovascular disease: an update.’ *Journal of the American college of cardiology*, *43*(10), pp.1731-1737.

Anderson, C., Majeste, A., Hanus, J. and Wang, S., 2016 ‘E-cigarette aerosol exposure induces reactive oxygen species, DNA damage, and cell death in vascular endothelial cells.’ *Toxicological Sciences*, *154*(2), pp.332-340.

Araújo, A.J.D., Menezes, A.M.B., Dórea, A.J.P.S., Torres, B.S., Viegas, C.A.D.A., Silva, C.A.R.D., Kirchenchtejn, C., Orfaliais, C.S., Sant'Anna, C.C., Botelho, C. and Gonçalves, C.M.C., 2004 ‘Diretrizes para cessação do tabagismo.’ *Jornal Brasileiro de Pneumologia*, *30*, pp.S1-S76.

ASH, 2021 ‘Use of E-Cigarettes (Vapes) among Adults in Great Britain.’

Auer, R., Schoeni, A., Humair, J.P., Jacot-Sadowski, I., Berlin, I., Stuber, M.J., Haller, M.L., Tango, R.C., Frei, A., Strassmann, A. and Bruggmann, P., 2024 ‘Electronic nicotine-delivery systems for smoking cessation.’ *New England Journal of Medicine*, *390*(7), pp.601-610.

Bajentri, A.L., Veeranna, N., Dixit, P.D. and Kulkarni, S.B., 2003 ‘Effect of 2-5 years of tobacco smoking on ventilatory function tests.’ *Journal of the Indian Medical Association*, *101*(2), pp.96-7.

Balady, G.J., Arena, R., Sietsema, K., Myers, J., Coke, L., Fletcher, G.F., Forman, D., Franklin, B., Guazzi, M., Gulati, M. and Keteyian, S.J., 2010 ‘Clinician’s guide to cardiopulmonary exercise testing in adults: a scientific statement from the American Heart Association.’ *Circulation*, *122*(2), pp.191-225.

Balkissoon, R. and Mkorombindo, T., 2022 ‘Journal Club: Impaired Ventilatory Efficiency and Exercise Intolerance in Former/Current Smokers With Dyspnea Disproportionate to Their Lung Function: Pathophysiological Insights Gained Through Cardiopulmonary Exercise Testing.’ *Chronic Obstructive Pulmonary Diseases: Journal of the COPD Foundation*, *9*(3), p.477.

Barberá, J.A., Peinado, V.I., Santos, S., Ramirez, J., Roca, J. and Rodriguez-Roisin, R., 2001 ‘Reduced expression of endothelial nitric oxide synthase in pulmonary arteries of smokers.’ *American journal of respiratory and critical care medicine*, *164*(4), pp.709-713.

Barbosa, G.W., Neder, J.A., Utida, K., O'Donnell, D.E. and de Tarso Müller, P., 2017 ‘Impaired exercise ventilatory efficiency in smokers with low transfer factor but normal spirometry.’ *European Respiratory Journal*, *49*(3).

Barua, R.S., Ambrose, J.A., Eales-Reynolds, L.J., DeVoe, M.C., Zervas, J.G. and Saha, D.C., 2001 ‘Dysfunctional endothelial nitric oxide biosynthesis in healthy smokers with impaired endothelium-dependent vasodilatation.’ *Circulation*, *104*(16), pp.1905-1910.

Barua, R.S., Ambrose, J.A., Srivastava, S., DeVoe, M.C. and Eales-Reynolds, L.J., 2003 ‘Reactive oxygen species are involved in smoking-induced dysfunction of nitric oxide biosynthesis and upregulation of endothelial nitric oxide synthase: an in vitro

demonstration in human coronary artery endothelial cells.' *Circulation*, *107*(18), pp.2342-2347.

Battista, L., Di Iorio, M., Tancredi, M., Acconcia, M.C., Torromeo, C., Barillà, F., Paravati, V., Gaudio, C. and Pannarale, G., 2013 'Cardiovascular effects of electronic cigarettes.'

Beaver, W.L., Wasserman, K.A.R.L.M.A.N. and Whipp, B.J., 1986 'A new method for detecting anaerobic threshold by gas exchange.' *Journal of applied physiology*, *60*(6), pp.2020-2027.

Beck, J., Sinderby, C., Lindström, L. and Grassino, A., 1998 'Effects of lung volume on diaphragm EMG signal strength during voluntary contractions.' *Journal of applied physiology*, *85*(3), pp.1123-1134.

Behar, R.Z., Davis, B., Wang, Y., Bahl, V., Lin, S. and Talbot, P., 2014 'Identification of toxicants in cinnamon-flavored electronic cigarette refill fluids.' *Toxicology in vitro*, *28*(2), pp.198-208.

Benowitz, N.L. and Fraiman, J.B., 2017 'Cardiovascular effects of electronic cigarettes.' *Nature Reviews Cardiology*, *14*(8), pp.447-456.

Bermudez, E.A., Rifai, N., Buring, J.E., Manson, J.E. and Ridker, P.M., 2002 'Relation between markers of systemic vascular inflammation and smoking in women.' *American Journal of Cardiology*, *89*(9), pp.1117-1119.

Bernaards, C.M., Twisk, J.W., Van Mechelen, W., Snel, J. and Kemper, H., 2003 'A longitudinal study on smoking in relationship to fitness and heart rate response.' *Medicine and science in sports and exercise*, *35*(5), pp.793-800.

Bernhard, D., Csordas, A., Henderson, B., Rossmann, A., Kind, M. and Wick, G., 2005 'Cigarette smoke metal-catalyzed protein oxidation leads to vascular endothelial cell contraction by depolymerization of microtubules.' *The FASEB journal*, *19*(9), pp.1096-1107.

Birajdar, Dr.G., Barawade, Dr.A., and Nagavekar, Dr.M., 2016 ‘Effect of smoking on maximum voluntary ventilation.’ IOSR Journal of Dental and Medical Sciences, 15(10), pp. 105–108.

Bland, J.M. and Altman, D., 1986 ‘Statistical methods for assessing agreement between two methods of clinical measurement.’ *The lancet*, *327*(8476), pp.307-310.

Bogaerts, K., Hubin, M., Van Diest, I., De Peuter, S., Van Houdenhove, B., Van Wambeke, P., Crombez, G. and Van den Bergh, O., 2007 ‘Hyperventilation in patients with chronic fatigue syndrome: the role of coping strategies.’ *Behaviour research and therapy*, *45*(11), pp.2679-2690.

Bohadana, A., Teculescu, D. and Martinet, Y., 2004 ‘Mechanisms of chronic airway obstruction in smokers.’ *Respiratory medicine*, *98*(2), pp.139-151.

Bolinder, G., Noren, A., Wahren, J. and De Faire, U., 1997 ‘Long-term use of smokeless tobacco and physical performance in middle-aged men.’ *European journal of clinical investigation*, *27*(5), pp.427-433.

Borg, G.A., 1982 ‘Psychophysical bases of perceived exertion.’ *Medicine and science in sports and exercise*, *14*(5), pp.377-381.

Bullen, C., 2008 ‘Impact of tobacco smoking and smoking cessation on cardiovascular risk and disease.’ *Expert review of cardiovascular therapy*, *6*(6), pp.883-895.

Calverley, P.M.A. and Koulouris, N.G., 2005 ‘Flow limitation and dynamic hyperinflation: key concepts in modern respiratory physiology.’ *European Respiratory Journal*, *25*(1), pp.186-199.

Caporale, A., Langham, M.C., Guo, W., Johncola, A., Chatterjee, S. and Wehrli, F.W., 2019 ‘Acute effects of electronic cigarette aerosol inhalation on vascular function detected at quantitative MRI.’ *Radiology*, *293*(1), p.97.

Cardellach, F., Alonso, J.R., López, S., Casademont, J. and Miró*, Ò., 2003 ‘Effect of smoking cessation on mitochondrial respiratory chain function.’ *Journal of Toxicology: Clinical Toxicology*, *41*(3), pp.223-228.

Carnevale, R., Sciarretta, S., Violi, F., Nocella, C., Loffredo, L., Perri, L., Peruzzi, M., Marullo, A.G., De Falco, E., Chimenti, I. and Valenti, V., 2016 ‘Acute impact of tobacco vs electronic cigarette smoking on oxidative stress and vascular function.’ *Chest*, *150*(3), pp.606-612.

Ceriello, A., 2008 ‘Possible role of oxidative stress in the pathogenesis of hypertension.’ *Diabetes care*, *31*, p.S181.

Chand, H.S., Muthumalage, T., Maziak, W. and Rahman, I., 2020 ‘Pulmonary toxicity and the pathophysiology of electronic cigarette, or vaping product, use associated lung injury.’ *Frontiers in pharmacology*, *10*, p.1619.

Chatterjee, M., Saluja, R., Kanneganti, S., Chinta, S. and Dikshit, M., 2007 ‘Biochemical and molecular evaluation of neutrophil NOS in spontaneously hypertensive rats.’ *Cellular and Molecular Biology*, *53*(1), pp.84-93.

Chaumont, M., Van De Borne, P., Bernard, A., Van Muylem, A., Deprez, G., Ullmo, J., Starczewska, E., Briki, R., De Hemptinne, Q., Zaher, W. and Debbas, N., 2019 ‘Fourth generation e-cigarette vaping induces transient lung inflammation and gas exchange disturbances: results from two randomized clinical trials.’ *American Journal of Physiology-Lung Cellular and Molecular Physiology*, *316*(5), pp.L705-L719.

Cheng, T., 2014 ‘Chemical evaluation of electronic cigarettes.’ *Tobacco control*, *23*(suppl 2), pp.ii11-ii17.

Clapp, P.W. and Jaspers, I., 2017 ‘Electronic cigarettes: their constituents and potential links to asthma.’ *Current allergy and asthma reports*, *17*, pp.1-13.

Clark, C.J., Cochrane, L.M., Mackay, E. and Paton, B., 2000 ‘Skeletal muscle strength and endurance in patients with mild COPD and the effects of weight training.’ *European Respiratory Journal*, *15*(1), pp.92-97.

Colberg, S.R., Casazza, G.A., Horning, M.A. and Brooks, G.A., 1995 ‘Metabolite and hormonal response in smokers during rest and sustained exercise.’ *Medicine and science in sports and exercise*, *27*(11), pp.1527-1534.

Come, C.E., Divo, M.J., Estépar, R.S.J., Sciurba, F.C., Criner, G.J., Marchetti, N., Scharf, S.M., Mosenifar, Z., Make, B.J., Keller, C.A. and Minai, O.A., 2012 ‘Lung deflation and oxygen pulse in COPD: results from the NETT randomized trial.’ *Respiratory medicine*, *106*(1), pp.109-119.

Constans, J. and Conri, C., 2006 ‘Circulating markers of endothelial function in cardiovascular disease.’ *Clinica chimica acta*, *368*(1-2), pp.33-47.

Cooper, K.H., Gey, G.O. and Bottenberg, R.A., 1968 ‘Effects of cigarette smoking on endurance performance.’ *Jama*, *203*(3), pp.189-192.

Cryer, P.E., Haymond, M.W., Santiago, J.V. and Shah, S.D., 1976 ‘Norepinephrine and epinephrine release and adrenergic mediation of smoking-associated hemodynamic and metabolic events.’ *New England journal of medicine*, *295*(11), pp.573-577.

Czernin, J. and Waldherr, C., 2003 ‘Cigarette smoking and coronary blood flow.’ *Progress in cardiovascular diseases*, *45*(5), pp.395-404.

Darabseh, M.Z., Maden-Wilkinson, T.M., Welbourne, G., Wüst, R.C., Ahmed, N., Aushah, H., Selfe, J., Morse, C.I. and Degens, H., 2021 ‘Fourteen days of smoking cessation improves muscle fatigue resistance and reverses markers of systemic inflammation.’ *Scientific reports*, *11*(1), p.12286.

de Oca, M.M., Loeb, E., Torres, S.H., De Sanctis, J., Hernández, N. and Tálamo, C., 2008 ‘Peripheral muscle alterations in non-COPD smokers.’ *Chest*, *133*(1), pp.13-18.

Deanfield, J.E. and Selwyn, A.P., 1986 ‘Character and causes of transient myocardial ischemia during daily life: Implications for treatment of patients with coronary disease.’ *The American Journal of Medicine*, *80*(4), pp.18-24.

Ekblom, B. and Huot, R., 1972 ‘Response to submaximal and maximal exercise at different levels of carboxyhemoglobin.’ *Acta physiologica scandinavica*, *86*(4), pp.474-482.

Elbehairy, A.F., Ciavaglia, C.E., Webb, K.A., Guenette, J.A., Jensen, D., Mourad, S.M., Neder, J.A. and O’Donnell, D.E., 2015 ‘Pulmonary gas exchange abnormalities in mild chronic obstructive pulmonary disease. Implications for dyspnea and exercise intolerance.’ *American journal of respiratory and critical care medicine*, *191*(12), pp.1384-1394.

Elbehairy, A.F., Guenette, J.A., Faisal, A., Ciavaglia, C.E., Webb, K.A., Jensen, D., Ramsook, A.H., Neder, J.A. and O'Donnell, D.E., 2016 ‘Mechanisms of exertional dyspnoea in symptomatic smokers without COPD.’ *European respiratory journal*, *48*(3), pp.694-705.

Elkalmi, R.M., Bhagavathul, A.S., Ya’u, A., Al-Dubai, S.A.R., Elsayed, T.M., Ahmad, A. and Mohamed, W., 2016 ‘Familiarity, perception, and reasons for electronic-cigarette experimentation among the general public in Malaysia: Preliminary insight.’ *Journal of Pharmacy and Bioallied Sciences*, *8*(3), pp.240-247.

Esen, A.M., Barutcu, I., Acar, M., Degirmenci, B., Kaya, D., Turkmen, M., Melek, M., Onrat, E., Esen, O.B. and Kirma, C., 2004 ‘Effect of smoking on endothelial function and wall thickness of brachial artery.’ *Circulation Journal*, *68*(12), pp.1123-1126.

Ezzati, M. and Lopez, A.D., 2003 ‘Estimates of global mortality attributable to smoking in 2000.’ *The lancet*, *362*(9387), pp.847-852.

Faisal, A., Beavers, K.R. and Hughson, R.L., 2010 ‘O2 uptake and blood pressure regulation at the onset of exercise: interaction of circadian rhythm and priming

exercise.' *American Journal of Physiology-Heart and Circulatory Physiology*, *299*(6), pp.H1832-H1842.

Faisal, A., Beavers, K.R. and Hughson, R.L., 2010 'O2 uptake and blood pressure regulation at the onset of exercise: interaction of circadian rhythm and priming exercise.' *American Journal of Physiology-Heart and Circulatory Physiology*, *299*(6), pp.H1832-H1842.

Farsalinos, K.E., Gillman, I.G., Melvin, M.S., Paolantonio, A.R., Gardow, W.J., Humphries, K.E., Brown, S.E., Poulas, K. and Voudris, V., 2015 'Nicotine levels and presence of selected tobacco-derived toxins in tobacco flavoured electronic cigarette refill liquids.' *International journal of environmental research and public health*, *12*(4), pp.3439-3452.

Faul, F., Erdfelder, E., Lang, A.-G., & Buchner, A., 2007 'G*Power 3: A flexible statistical power analysis program for the social, behavioral, and biomedical sciences.' *Behavior Research Methods*, *39*, 175-191.

Fetterman, J.L., Weisbrod, R.M., Feng, B., Bastin, R., Tuttle, S.T., Holbrook, M., Baker, G., Robertson, R.M., Conklin, D.J., Bhatnagar, A. and Hamburg, N.M., 2018 'Flavorings in tobacco products induce endothelial cell dysfunction.' *Arteriosclerosis, thrombosis, and vascular biology*, *38*(7), pp.1607-1615.

Fillon, M., 2015 'Electronic cigarettes may lead to nicotine addiction.' *JNCI Journal of the National Cancer Institute, 107*(3), pp.1-8

Fletcher, C.M., 1975 'The natural history of chronic bronchitis.' *Community Health*, *7*(2), pp.70-78.

Franzen, K.F., Willig, J., Cayo Talavera, S., Meusel, M., Sayk, F., Reppel, M., Dalhoff, K., Mortensen, K. and Droemann, D., 2018 'E-cigarettes and cigarettes worsen peripheral and central hemodynamics as well as arterial stiffness: A randomized, double-blinded pilot study.' *Vascular medicine*, *23*(5), pp.419-425

Frazão, M., Silva, P.E., Frazão, W., da Silva, V.Z.M., de Valois Correia Jr, M.A. and Neto, M.G., 2019 'Dynamic hyperinflation impairs cardiac performance during exercise in COPD.' *Journal of cardiopulmonary rehabilitation and prevention*, *39*(3), pp.187-192.

Gagnon, P., Lemire, B.B., Dubé, A., Saey, D., Porlier, A., Croteau, M., Provencher, S., Debigaré, R. and Maltais, F., 2014 'Preserved function and reduced angiogenesis potential of the quadriceps in patients with mild COPD.' *Respiratory research*, *15*(1), pp.1-9.

Gandevia, B. and Hugh-Jones, P., 1957 'Terminology for measurements of ventilatory capacity: a report to the thoracic society.' *Thorax*, *12*(4), p.290.

Gandevia, S.C., 1988 'Neural mechanisms underlying the sensation of breathlessness: kinesthetic parallels between respiratory and limb muscles.' *Australian and New Zealand journal of medicine*, *18*(1), pp.83-91.

Gauthier, A.P., Verbanck, S.Y.L.V.I.A., Estenne, M.A.R.C., Segebarth, C.H.R.I.S.T.O.P.H.E.R., Macklem, P.T. and Paiva, M.A.N.U.E.L., 1994 'Three-dimensional reconstruction of the in vivo human diaphragm shape at different lung volumes.' *Journal of applied physiology*, *76*(2), pp.495-506.

Gerloff, J., Sundar, I.K., Freter, R., Sekera, E.R., Friedman, A.E., Robinson, R., Pagano, T. and Rahman, I., 2017 'Inflammatory response and barrier dysfunction by different e-cigarette flavoring chemicals identified by gas chromatography–mass spectrometry in e-liquids and e-vapors on human lung epithelial cells and fibroblasts.' *Applied in vitro toxicology*, *3*(1), pp.28-40.

Gläser, S., Obst, A., Opitz, C.F., Dörr, M., Felix, S.B., Empen, K., Völzke, H., Ewert, R., Schäper, C. and Koch, B., 2011 'Peripheral endothelial dysfunction is associated with gas exchange inefficiency in smokers.' *Respiratory research*, *12*, pp.1-9.

Goldbarg, A.N., Krone, R.J. and Resnekov, L., 1971 ‘Effects of cigarette smoking on hemodynamics at rest and during exercise. I. Normal subjects.’ *Chest*, *60*(6), pp.531-536.

Goniewicz, M.L., Knysak, J., Gawron, M., Kosmider, L., Sobczak, A., Kurek, J., Prokopowicz, A., Jablonska-Czapla, M., Rosik-Dulewska, C., Havel, C. and Jacob, P., 2014 ‘Levels of selected carcinogens and toxicants in vapour from electronic cigarettes.’ *Tobacco control*, *23*(2), pp.133-139.

Gordon, C., Gudi, K., Krause, A., Sackrowitz, R., Harvey, B.G., Strulovici-Barel, Y., Mezey, J.G. and Crystal, R.G., 2011 ‘Circulating endothelial microparticles as a measure of early lung destruction in cigarette smokers.’ *American journal of respiratory and critical care medicine*, *184*(2), pp.224-232.

Graham, B.L., Steenbruggen, I., Miller, M.R., Barjaktarevic, I.Z., Cooper, B.G., Hall, G.L., Hallstrand, T.S., Kaminsky, D.A., McCarthy, K., McCormack, M.C. and Oropez, C.E., 2019 ‘Standardization of spirometry 2019 update. An official American thoracic society and European respiratory society technical statement.’ *American journal of respiratory and critical care medicine*, *200*(8), pp.e70-e88.

Grewal, J., McCully, R.B., Kane, G.C., Lam, C. and Pellikka, P.A., 2009 ‘Left ventricular function and exercise capacity.’ *Jama*, *301*(3), pp.286-294.

Guenette, J.A., Chin, R.C., Cheng, S., Dominelli, P.B., Raghavan, N., Webb, K.A., Neder, J.A. and O’Donnell, D.E., 2014 ‘Mechanisms of exercise intolerance in global initiative for chronic obstructive lung disease grade 1 COPD.’ *European Respiratory Journal*, *44*(5), pp.1177-1187.

Hagg, U., Wandt, B., Bergstrom, G., Volkmann, R. and Gan, L.M., 2005 ‘Physical exercise capacity is associated with coronary and peripheral vascular function in healthy young adults.’ *American Journal of Physiology-Heart and Circulatory Physiology*, *289*(4), pp.H1627-H1634.

Hansen, J.E., Sue, D.Y. and Wasserman, K., 1984 ‘Predicted values for clinical exercise testing.’ *American Review of Respiratory Disease*, *129*(2P2), pp.S49-S55.

Hansson, L.E.N.N.A.R.T., Choudry, N.B., Karlsson, J.A. and Fuller, R.W., 1994 ‘Inhaled nicotine in humans: effect on the respiratory and cardiovascular systems.’ *Journal of Applied Physiology*, *76*(6), pp.2420-2427.

Haptonstall, K.P., Choroomi, Y., Moheimani, R., Nguyen, K., Tran, E., Lakhani, K., Ruedisueli, I., Gornbein, J. and Middlekauff, H.R., 2020 ‘Differential effects of tobacco cigarettes and electronic cigarettes on endothelial function in healthy young people.’ *American Journal of Physiology-Heart and Circulatory Physiology*, *319*(3), pp.H547-H556.

Hartman, C.L. and Ford, D.A., 2018 ‘MPO (Myeloperoxidase) Caused Endothelial Dysfunction: Not So Positive It Is About the Bleach, It May Be a Fatal Attraction.’ *Arteriosclerosis, thrombosis, and vascular biology*, *38*(8), pp.1676-1677.

Hayano, J., Yamada, M., Sakakibara, Y., Fujinami, T., Yokoyama, K., Watanabe, Y. and Takata, K., 1990 ‘Short-and long-term effects of cigarette smoking on heart rate variability.’ *The American journal of cardiology*, *65*(1), pp.84-88.

Hering, D., Somers, V.K., Kara, T., Kucharska, W., Jurak, P., Bieniaszewski, L. and Narkiewicz, K., 2006 ‘Sympathetic neural responses to smoking are age dependent.’ *Journal of hypertension*, *24*(4), pp.691-695.

Hirai, N., Kawano, H., Hirashima, O., Motoyama, T., Moriyama, Y., Sakamoto, T., Kugiyama, K., Ogawa, H., Nakao, K. and Yasue, H., 2000 ‘Insulin resistance and endothelial dysfunction in smokers: effects of vitamin C.’ *American Journal of Physiology-Heart and Circulatory Physiology*, *279*(3), pp.H1172-H1178.

Hirsch, G.L., Sue, D.Y., Wasserman, K.A.R.L.M.A.N., Robinson, T.E. and Hansen, J.E., 1985 ‘Immediate effects of cigarette smoking on cardiorespiratory responses to exercise.’ *Journal of Applied Physiology*, *58*(6), pp.1975-1981.

Hueper, K., Vogel-Claussen, J., Parikh, M.A., Austin, J.H., Bluemke, D.A., Carr, J., Choi, J., Goldstein, T.A., Gomes, A.S., Hoffman, E.A. and Kawut, S.M., 2015 'Pulmonary microvascular blood flow in mild chronic obstructive pulmonary disease and emphysema.' The MESA COPD Study. *American journal of respiratory and critical care medicine*, *192*(5), pp.570-580.

Huie, M.J., Casazza, G.A., Horning, M.A. and Brooks, G.A., 1996 'Smoking increases conversion of lactate to glucose during submaximal exercise.' *Journal of applied physiology*, *80*(5), pp.1554-1559.

Hutzler, C., Paschke, M., Kruschinski, S., Henkler, F., Hahn, J. and Luch, A., 2014 'Chemical hazards present in liquids and vapors of electronic cigarettes.' *Archives of toxicology*, *88*, pp.1295-1308.

Inaba, Y., Chen, J.A. and Bergmann, S.R., 2010 'Prediction of future cardiovascular outcomes by flow-mediated vasodilatation of brachial artery: a meta-analysis.' *The international journal of cardiovascular imaging*, *26*, pp.631-640.

Ishida, T., Pinkerton, K.E. and Takeuchi, M., 2009 'Alveolar macrophage from cigarette smoke-exposed mice inhibits B lymphocyte proliferation stimulated with LPS.' *Respiration*, *77*(1), pp.91-95.

Jerram, J. and Bendel, N. (no date) '*Smoke-free Manchester - our plan for Tobacco Control 2018-2021'* (online) (Accessed on 14th August 2024) https://www.readkong.com/page/smoke-free-manchester-our-plan-for-tobacco-control-2164556

Jha, P., Ramasundarahettige, C., Landsman, V., Rostron, B., Thun, M., Anderson, R.N., McAfee, T. and Peto, R., 2013 '21st-century hazards of smoking and benefits of cessation in the United States.' *New England Journal of Medicine*, *368*(4), pp.341-350.

Jobse, B.N., McCurry, C.A., Morissette, M.C., Rhem, R.G., Stämpfli, M.R. and Labiris, N.R., 2014 'Impact of inflammation, emphysema, and smoking cessation on V/Q in mouse models of lung obstruction.' *Respiratory research*, *15*, pp.1-9.

Johnson, H.M., Gossett, L.K., Piper, M.E., Aeschlimann, S.E., Korcarz, C.E., Baker, T.B., Fiore, M.C. and Stein, J.H., 2010 'Effects of smoking and smoking cessation on endothelial function: 1-year outcomes from a randomized clinical trial.' *Journal of the American College of Cardiology*, *55*(18), pp.1988-1995.

Kailas, M., Layton, A., Pesce, M., Garofano, R., Starc, T., Berman-Rosenzweig, E., Liberman, L. and Krishnan, U., 2021 'Exercise-Induced ECG Changes Predict Adverse Outcomes in Pulmonary Hypertension.' *Circulation*, *144*(Suppl_1), pp.A10753-A10753.

Karia, R.M., 2012 'Comparative study of peak expiratory flow rate and maximum voluntary ventilation between smokers and non-smokers.' *National J Med Res*, *2*, pp.191-3.

Kim, W.D., Eidelman, D.H., Izquierdo, J.L., Ghezzo, H., Saetta, M.P. and Cosio, M.G., 1991 'Centrilobular and panlobular emphysema in smokers.' *Am Rev Respir Dis*, *144*, pp.1385-1390.

Kirby, M., Owrangi, A., Svenningsen, S., Wheatley, A., Coxson, H.O., Paterson, N.A., McCormack, D.G. and Parraga, G., 2013 'On the role of abnormal DLCO in ex-smokers without airflow limitation: symptoms, exercise capacity and hyperpolarised helium-3 MRI.' *Thorax*, *68*(8), pp.752-759.

Kizhakke Puliyakote, A.S., Elliott, A.R., Sá, R.C., Anderson, K.M., Crotty Alexander, L.E. and Hopkins, S.R., 2021 'Vaping disrupts ventilation-perfusion matching in asymptomatic users.' *Journal of Applied Physiology*, *130*(2), pp.308-317.

Kjeldsen, K., Astrup, P. and Wanstrup, J., 1969 'Reversal of rabbit atheromatosis by hyperoxia.' *Journal of Atherosclerosis Research*, *10*(2), pp.173-178.

Kobayashi, Y., Takeuchi, T., Hosoi, T. and Loeppky, J.A., 2004 ‘Effects of habitual smoking on cardiorespiratory responses to sub-maximal exercise.’ *Journal of physiological anthropology and applied human science*, *23*(5), pp.163-169.

Kuperman, A.S. and Riker, J.B., 1973 ‘The variable effect of smoking on pulmonary function.’ *Chest*, *63*(5), pp.655-660.

Lange, P., Groth, S., Nyboe, G.J., Mortensen, J., Appleyard, M., Jensen, G. and Schnohr, P., 1989 ‘Effects of smoking and changes in smoking habits on the decline of FEV1.’ *European Respiratory Journal*, *2*(9), pp.811-816.

Lauer, M.S., 2004 ‘Chronotropic incompetence: ready for prime time.’ *Journal of the American College of Cardiology*, *44*(2), pp.431-432.

Lauer, M.S., Okin, P.M., Larson, M.G., Evans, J.C. and Levy, D., 1996 ‘Impaired heart rate response to graded exercise: prognostic implications of chronotropic incompetence in the Framingham Heart Study.’ *Circulation*, *93*(8), pp.1520-1526.

Lauer, M.S., Pashkow, F.J., Larson, M.G. and Levy, D., 1997 ‘Association of cigarette smoking with chronotropic incompetence and prognosis in the Framingham Heart Study.’ *Circulation*, *96*(3), pp.897-903.

Laustiola, K.E., Lassila, R., Kaprio, J. and Koskenvuo, M., 1988 ‘Decreased beta-adrenergic receptor density and catecholamine response in male cigarette smokers.’ A study of monozygotic twin pairs discordant for smoking. *Circulation*, *78*(5), pp.1234-1240.

Lavi, S., Prasad, A., Yang, E.H., Mathew, V., Simari, R.D., Rihal, C.S., Lerman, L.O. and Lerman, A., 2007 ‘Smoking is associated with epicardial coronary endothelial dysfunction and elevated white blood cell count in patients with chest pain and early coronary artery disease.’ *Circulation*, *115*(20), pp.2621-2627.

Leigh, N.J., Lawton, R.I., Hershberger, P.A. and Goniewicz, M.L., 2016 ‘Flavourings significantly affect inhalation toxicity of aerosol generated from electronic nicotine delivery systems (ENDS).’ *Tobacco control*, *25*(Suppl 2), pp.ii81-ii87.

Levy, D.T., Warner, K.E., Cummings, K.M., Hammond, D., Kuo, C., Fong, G.T., Thrasher, J.F., Goniewicz, M.L. and Borland, R., 2019 ‘Examining the relationship of vaping to smoking initiation among US youth and young adults: a reality check.’ *Tobacco control*, *28*(6), pp.629-635.

Lifshay, A.L.A.N., Fast, C.W. and Glazier, J.B., 1971 ‘Effects of changes in respiratory pattern on physiological dead space.’ *Journal of Applied Physiology*, *31*(3), pp.478-483.

Li, S., Hoskins, P.R., Anderson, T. and McDicken, W.N., 1993 ‘Measurement of mean velocity during pulsatile flow using time-averaged maximum frequency of Doppler ultrasound waveforms.’ *Ultrasound in medicine & biology*, *19*(2), pp.105-113.

Lucini, D., Bertocchi, F., Malliani, A. and Pagani, M., 1996 ‘A controlled study of the autonomic changes produced by habitual cigarette smoking in healthy subjects.’ *Cardiovascular research*, *31*(4), pp.633-639.

Markuljak, I., Ivankova, J. and Kubisz, P., 1995 ‘Thrombomodulin and von Willebrand factor in smokers and during smoking.’ *Nouvelle revue française d'hématologie*, *37*(2), pp.137-139.

Mayhan, W.G. and Sharpe, G.M., 1999 ’Chronic exposure to nicotine alters endothelium-dependent arteriolar dilatation: effect of superoxide dismutase.’ *Journal of applied physiology*, *86*(4), pp.1126-1134.

McCully, K.K. and Faulkner, J.A., 1983 ‘Length-tension relationship of mammalian diaphragm muscles.’ *Journal of Applied Physiology*, *54*(6), pp.1681-1686.

McDonough, P. and Moffatt, R.J., 1999 ‘Smoking-induced elevations in blood carboxyhaemoglobin levels: Effect on maximal oxygen uptake.’ *Sports Medicine*, *27*, pp.275-283.

McNeill, A., Brose, L.S., Calder, R., Hitchman, S.C., Hajek, P. and McRobbie, H., 2015 ‘E-cigarettes: an evidence update.’ *Public Health England*, *3*(6), pp.14-15.

Mehta, H., Nazzal, K. and Sadikot, R.T., 2008 ‘Cigarette smoking and innate immunity.’ *Inflammation Research*, *57*, pp.497-503.

Melliti, W., Kammoun, R., Masmoudi, D., Ahmaidi, S., Masmoudi, K., Alassery, F., Hamam, H. and Chlif, M., 2021 ‘Effect of Six-Minute Walk Test and Incremental Exercise on Inspiratory Capacity, Ventilatory Constraints, Breathlessness and Exercise Performance in Sedentary Male Smokers without Airway Obstruction.’ *International Journal of Environmental Research and Public Health*, *18*(23), p.12665.

Meo, S.A., Ansary, M.A., Barayan, F.R., Almusallam, A.S., Almehaid, A.M., Alarifi, N.S., Alsohaibani, T.A. and Zia, I., 2019 ‘Electronic cigarettes: impact on lung function and fractional exhaled nitric oxide among healthy adults.’ *American Journal of Men's health*, *13*(1), p.1557988318806073.

Mercado, C. and Jaimes, E.A., 2007 ‘Cigarette smoking as a risk factor for atherosclerosis and renal disease: novel pathogenic insights.’ *Current hypertension reports*, *9*(1), pp.66-72.

Miller, M.R., Hankinson, J.A.T.S., Brusasco, V., Burgos, F., Casaburi, R., Coates, A., Crapo, R., Enright, P., Van Der Grinten, C.P.M., Gustafsson, P. and Jensen, R., 2005 ‘Standardisation of spirometry.’ *European respiratory journal*, *26*(2), pp.319-338.

Mohammadi, L., Han, D.D., Xu, F., Huang, A., Derakhshandeh, R., Rao, P., Whitlatch, A., Cheng, J., Keith, R.J., Hamburg, N.M. and Ganz, P., 2022 ‘Chronic E-

cigarette use impairs endothelial function on the physiological and cellular levels.' *Arteriosclerosis, thrombosis, and vascular biology*, *42*(11), pp.1333-1350.

Moheimani, R.S., Bhetraratana, M., Peters, K.M., Yang, B.K., Yin, F., Gornbein, J., Araujo, J.A. and Middlekauff, H.R., 2017 'Sympathomimetic effects of acute e-cigarette use: role of nicotine and non-nicotine constituents.' *Journal of the American Heart Association*, *6*(9), p.e006579.

Morrow, J.D., Frei, B., Longmire, A.W., Gaziano, J.M., Lynch, S.M., Shyr, Y., Strauss, W.E., Oates, J.A. and Roberts, L.J., 1995 'Increase in circulating products of lipid peroxidation (F2-isoprostanes) in smokers—smoking as a cause of oxidative damage.' *New England Journal of Medicine*, *332*(18), pp.1198-1203.

Morse, C.I., Wüst, R.C., Jones, D.A., De Haan, A. and Degens, H., 2007 'Muscle fatigue resistance during stimulated contractions is reduced in young male smokers.' *Acta physiologica*, *191*(2), pp.123-129.

Morton, A.R. and Holmik, E.V., 1985 'The effects of cigarette smoking on maximal oxygen consumption and selected physiological responses of elite team sportsmen.' *European journal of applied physiology and occupational physiology*, *53*(4), pp.348-352.

Murohara, T.O.Y.O.A.K.I., Kugiyama, K.I.Y.O.T.A.K.A., Ohgushi, M.A.S.A.M.I.C.H.I., Sugiyama, S.E.I.G.O. and Yasue, H.I.R.O.F.U.M.I., 1994 'Cigarette smoke extract contracts isolated porcine coronary arteries by superoxide anion-mediated degradation of EDRF.' *American Journal of Physiology-Heart and Circulatory Physiology*, *266*(3), pp.H874-H880.

Myers, J. and Ashley, E., 1997 'Dangerous curves: a perspective on exercise, lactate, and the anaerobic threshold.' *Chest*, *111*(3), pp.787-795.

Naeije, R., 2014 'Lung mechanics and exertional dyspnoea in pulmonary arterial hypertension.' *Respiration*, *88*(1), pp.16-17.

Narkiewicz, K., Van De Borne, P.J., Hausberg, M., Cooley, R.L., Winniford, M.D., Davison, D.E. and Somers, V.K., 1998 ‘Cigarette smoking increases sympathetic outflow in humans.’ *Circulation*, *98*(6), pp.528-534.

Neder, J.A. and O’Donnell, D.E., 2015 ‘Pulmonary gas exchange abnormalities in mild chronic obstructive pulmonary disease. Implications for dyspnea and exercise intolerance.’ *American journal of respiratory and critical care medicine*, *191*(12), pp.1384-1394.

Neder, J.A., Andreoni, S., Lerario, M.C. and Nery, L.E., 1999 ‘Reference values for lung function tests: II. Maximal respiratory pressures and voluntary ventilation.’ *Brazilian journal of medical and biological research*, *32*, pp.719-727.

Notley, C., Varley, A., Pope, I., Dawkins, L. and Ward, E., 2024 ‘Young people’s use of disposable vapes: A qualitative study.’ *Addiction*, pp.1-10

Nowak, D., Antczak, A., Krol, M., Pietras, T., Shariati, B., Bialasiewicz, P., Jeczkowski, K. and Kula, P., 1996 ‘Increased content of hydrogen peroxide in the expired breath of cigarette smokers.’ *European Respiratory Journal*, *9*(4), pp.652-657.

O'Donnell, D.E. and Laveneziana, P., 2006 ‘The clinical importance of dynamic lung hyperinflation in COPD.’ *COPD: Journal of Chronic Obstructive Pulmonary Disease*, *3*(4), pp.219-232.

O'Donnell, D.E., Bertley, J.C., Chau, L.K. and Webb, K.A., 1997 ‘Qualitative aspects of exertional breathlessness in chronic airflow limitation: pathophysiologic mechanisms.’ *American journal of respiratory and critical care medicine*, *155*(1), pp.109-115.

O'Donnell, D.E., Guenette, J.A., Maltais, F. and Webb, K.A., 2012 ‘Decline of resting inspiratory capacity in COPD: the impact on breathing pattern, dyspnea, and ventilatory capacity during exercise.’ *Chest*, *141*(3), pp.753-762.

O'donnell, D.E., Revill, S.M. and Webb, K.A., 2001 ‘Dynamic hyperinflation and exercise intolerance in chronic obstructive pulmonary disease.’ *American journal of respiratory and critical care medicine*, *164*(5), pp.770-777.
O’Donnell, D.E., 2016 ‘Common mechanisms of dyspnea in chronic interstitial and obstructive lung disorders.’ *American journal of respiratory and critical care medicine*, *193*(3), pp.299-309.

Olfert, I.M., DeVallance, E., Hoskinson, H., Branyan, K.W., Clayton, S., Pitzer, C.R., Sullivan, D.P., Breit, M.J., Wu, Z., Klinkhachorn, P. and Mandler, W.K., 2018 ‘Chronic exposure to electronic cigarettes results in impaired cardiovascular function in mice.’ *Journal of applied physiology*, *124*(3), pp.573-582.

ÖRLANDER, J., KIESSLING, K.H. and LARSSON, L., 1979 ‘Skeletal muscle metabolism, morphology and function in sedentary smokers and nonsmokers.’ *Acta Physiologica Scandinavica*, *107*(1), pp.39-46.

Padmavathy, K.M., 2008. Comparative study of pulmonary function variables in relation to type of smoking. *Indian J Physiol Pharmacol*, *52*(2), pp.193-196. K.M., 2008 ‘Comparative study of pulmonary function variables in relation to type of smoking.’ *Indian J Physiol Pharmacol*, *52*(2), pp.193-196.

Papathanasiou, G., Georgakopoulos, D., Georgoudis, G., Spyropoulos, P., Perrea, D. and Evangelou, A., 2007 ‘Effects of chronic smoking on exercise tolerance and on heart rate-systolic blood pressure product in young healthy adults.’ *European Journal of Preventive Cardiology*, *14*(5), pp.646-652.

Papathanasiou, G., Georgakopoulos, D., Papageorgiou, E., Zerva, E., Michalis, L., Kalfakakou, V. and Evangelou, A., 2013 ‘Effects of smoking on heart rate at rest and during exercise, and on heart rate recovery, in young adults.’ *Hellenic J Cardiol*, *54*(3), pp.168-77.

Papathanasiou, G., Mamali, A., Papafloratos, S. and Zerva, E., 2014 ‘Effects of smoking on

cardiovascular function: the role of nicotine and carbon monoxide.' *Health science journal*, *8*(2), p.274.

Parker, B.A., Trehearn, T.L. and Meendering, J.R., 2009 'Pick your Poiseuille: normalizing the shear stimulus in studies of flow-mediated dilation.' *Journal of Applied Physiology*, *107*(4), pp.1357-1359.

Parshall, M.B., Schwartzstein, R.M., Adams, L., Banzett, R.B., Manning, H.L., Bourbeau, J., Calverley, P.M., Gift, A.G., Harver, A., Lareau, S.C. and Mahler, D.A., 2012 'An official American Thoracic Society statement: update on the mechanisms, assessment, and management of dyspnea.' *American journal of respiratory and critical care medicine*, *185*(4), pp.435-452.

Pellegrino, R., Brusasco, V., Rodarte, J.R. and Babb, T.G., 1993 'Expiratory flow limitation and regulation of end-expiratory lung volume during exercise.' *Journal of applied physiology*, *74*(5), pp.2552-2558.

Petersen, A.M.W., Magkos, F., Atherton, P., Selby, A., Smith, K., Rennie, M.J., Pedersen, B.K. and Mittendorfer, B., 2007 'Smoking impairs muscle protein synthesis and increases the expression of myostatin and MAFbx in muscle.' *American Journal of Physiology-Endocrinology and Metabolism*, 293(3), pp.E843–E84.

Pirnay, F., Dujardin, J., Deroanne, R. and Petit, J.M., 1971 'Muscular exercise during intoxication by carbon monoxide.' *Journal of Applied Physiology*, *31*(4), pp.573-575.

Piqueras, M.C. and Cosio, M.G., 2001 'Disease of the airways in chronic obstructive pulmonary disease.' *European Respiratory Journal*, *18*(34 suppl), pp.41s-49s.

Polkey, M.I., Green, M. and Moxham, J., 1995 'Measurement of respiratory muscle strength.' *Thorax*, *50*(11), p.1131.

Polosa, R., Cibella, F., Caponnetto, P., Maglia, M., Prosperini, U., Russo, C. and Tashkin, D., 2017 ‘Health impact of E-cigarettes: a prospective 3.5-year study of regular daily users who have never smoked.’ *Scientific reports*, *7*(1), p.13825.

Poredoš, P., Orehek, M., Tratnik, E. and Poredoš, P., 1999 ‘Smoking is associated with dose-related increase of intima-media thickness and endothelial dysfunction.’ *Angiology*, *50*(3), pp.201-208.

Pucci, M.L., Lin, L.A.N.G. and Nasjletti, A., 1992 ‘Pressor and renal vasoconstrictor effects of NG-nitro-L-arginine as affected by blockade of pressor mechanisms mediated by the sympathetic nervous system, angiotensin, prostanoids and vasopressin.’ *Journal of Pharmacology and Experimental Therapeutics*, *261*(1), pp.240-245.

Putzhammer, R., Doppler, C., Jakschitz, T., Heinz, K., Förste, J., Danzl, K., Messner, B. and Bernhard, D., 2016 ‘Vapours of US and EU market leader electronic cigarette brands and liquids are cytotoxic for human vascular endothelial cells.’ *PloS one*, *11*(6), p.e0157337.

Qasim, H., Karim, Z.A., Rivera, J.O., Khasawneh, F.T. and Alshbool, F.Z., 2017 ‘Impact of electronic cigarettes on the cardiovascular system.’ *Journal of the American Heart Association*, *6*(9), p.e006353.

Quanjer, P.H., Stanojevic, S., Cole, T.J., Baur, X., Hall, G.L., Culver, B.H., Enright, P.L., Hankinson, J.L., Ip, M.S., Zheng, J. and Stocks, J., 2012 ‘Multi-ethnic reference values for spirometry for the 3–95-yr age range: the global lung function 2012 equations.’ *European Respiratory Journal,* 40(6), pp.1324-1343.

Quillen, J.E., Rossen, J.D., Oskarsson, H.J., Minor Jr, R.L., Lopez, J.A.G. and Winniford, M.D., 1993 ‘Acute effect of cigarette smoking on the coronary circulation: constriction of epicardial and resistance vessels.’ *Journal of the American College of Cardiology*, *22*(3), pp.642-647.

Rainoldi, A., Bullock-Saxton, J.E., Cavarretta, F. and Hogan, N., 2001 'Repeatability of maximal voluntary force and of surface EMG variables during voluntary isometric contraction of quadriceps muscles in healthy subjects.' *Journal of Electromyography and Kinesiology*, *11*(6), pp.425-438.

Regan, E.A., Lynch, D.A., Curran-Everett, D., Curtis, J.L., Austin, J.H., Grenier, P.A., Kauczor, H.U., Bailey, W.C., DeMeo, D.L., Casaburi, R.H. and Friedman, P., 2015 'Clinical and radiologic disease in smokers with normal spirometry.' *JAMA internal medicine*, *175*(9), pp.1539-1549.

Reinders, J.H., Brinkman, H.J., van Mourik, J.A. and de Groot, P.G., 1986 'Cigarette smoke impairs endothelial cell prostacyclin production.' *Arteriosclerosis: An Official Journal of the American Heart Association, Inc.*, *6*(1), pp.15-23.

Robinson, Chelsea N., 2020 'The impact of e-cigarette smoking on pulmonary responses to maximal exercise.' M.Sc. James Madison University. (online) (Accessed 16th August) https://commons.lib.jmu.edu/cgi/viewcontent.cgi?article=1048&context=masters202029

Rooks, C.R., McCully, K.K. and Dishman, R.K., 2011 'Acute exercise improves endothelial function despite increasing vascular resistance during stress in smokers and nonsmokers.' *Psychophysiology*, *48*(9), pp.1299-1308.

Rotstein, A., Sagiv, M., Yaniv-Tamir, A., Fisher, N. and Dotan, R., 1991 'Smoking effect on exercise response kinetics of oxygen uptake and related variables.' *International journal of sports medicine*, *12*(03), pp.281-284.

Sadaka, A.S., Faisal, A., Khalil, Y.M., Mourad, S.M., Zidan, M.H., Polkey, M.I. and Hopkinson, N.S., 2021 'Reduced skeletal muscle endurance and ventilatory efficiency during exercise in adult smokers without airflow obstruction.' *Journal of Applied Physiology*, *130*(4), pp.976-986.

Sandvik, L., Erikssen, J., Ellestad, M., Erikssen, G., Thaulow, E., Mundal, R. and Rodahl, K., 1995 'Heart rate increase and maximal heart rate during exercise as predictors of cardiovascular mortality: a 16-year follow-up study of 1960 healthy men.' *Coronary artery disease*, *6*(8), pp.667-679.

Savonen, K.P., Lakka, T.A., Laukkanen, J.A., Halonen, P.M., Rauramaa, T.H., Salonen, J.T. and Rauramaa, R., 2006 'Heart rate response during exercise test and cardiovascular mortality in middle-aged men.' *European heart journal*, *27*(5), pp.582-588.

Shahandeh, N., Chowdhary, H. and Middlekauff, H.R., 2021 'Vaping and cardiac disease.' *Heart*, *107*(19), pp.1530-1535.

Silva, C.G. and Araújo, C.G.S., 2015 'Sex-specific equations to estimate maximum oxygen uptake in cycle ergometry.' *Arquivos Brasileiros de Cardiologia*, *105*, pp.381-389.

Simpson, A.J., Gray, R.S., Moore, N.R. and Booth, N.A., 1997 'The effects of chronic smoking on the fibrinolytic potential of plasma and platelets.' *British journal of haematology*, *97*(1), pp.208-213.

Sinderby, C., Spahija, J., Beck, J., Kaminski, D., Yan, S., Comtois, N. and Sliwinski, P., 2001 'Diaphragm activation during exercise in chronic obstructive pulmonary disease.' *American journal of respiratory and critical care medicine*, *163*(7), pp.1637-1641.

Skotsimara, G., Antonopoulos, A., Oikonomou, E., Ioakeimidis, N., Tsalamandris, S., Georgakopoulos, C., Vogiatzi, G., Koliostasis, L., Siasos, G., Vlachopoulos, C. and

Tousoulis, D., 2018 'Exposure to e-cigarette adversely affects blood pressure and heart rate in healthy individuals: A Systematic review and meta-analysis.' *Circulation*, *138*(Suppl_1), pp.A11507-A11507.

Soffler, M.I., Hayes, M.M. and Schwartzstein, R.M., 2017 ‘Respiratory sensations in dynamic hyperinflation: physiological and clinical applications.’ *Respiratory Care*, *62*(9), pp.1212-1223.

Sopori, M., 2002 ‘Effects of cigarette smoke on the immune system.’ *Nature Reviews Immunology*, *2*(5), pp.372-377.

Soumagne, T., Guillien, A., Roche, N., Dalphin, J.C. and Degano, B., 2020 ‘Never-smokers with occupational COPD have better exercise capacities and ventilatory efficiency than matched smokers with COPD.’ *Journal of Applied Physiology*, *129*(6), pp.1257-1266.

Stockley, J., Sapey, E., Gompertz, S., Edgar, R. and Cooper, B., 2018 ‘Pilot data of the short-term effects of e-cigarrette vaping on lung function.’

Suhara, T., Fukuo, K., Sugimoto, T., Morimoto, S., Nakahashi, T., Hata, S., Shimizu, M. and Ogihara, T., 1998 ‘Hydrogen peroxide induces up-regulation of Fas in human endothelial cells.’ *The Journal of Immunology*, *160*(8), pp.4042-4047.

Suminski, R.R., Wier, L.T., Poston, W., Arenare, B., Randles, A. and Jackson, A.S., 2009 ‘The effect of habitual smoking on measured and predicted VO2max.’ *Journal of Physical Activity and Health*, *6*(5), pp.667-673.

Szmitko, P.E., Wang, C.H., Weisel, R.D., de Almeida, J.R., Anderson, T.J. and Verma, S., 2003 ‘New markers of inflammation and endothelial cell activation: Part I.’ *Circulation*, *108*(16), pp.1917-1923.

Tachmes, L., Fernandez, R.J. and Sackner, M.A., 1978 ‘Hemodynamic effects of smoking cigarettes of high and low nicotine content.’ *Chest*, *74*(3), pp.243-246.

Takeshita, D., Nakajima-Takenaka, C., Shimizu, J., Hattori, H., Nakashima, T., Kikuta, A., Matsuyoshi, H. and Takaki, M., 2009 ‘Effects of formaldehyde on cardiovascular system in in situ rat hearts.’ *Basic & clinical pharmacology & toxicology*, *105*(4), pp.271-280.

TANI, T., KOGI, K. and HORIGUCHI, Y., 1986 ‘Inhibitory effects of formaldehyde inhalation on the cardiovascular and respiratory systems in unanesthetized rabbits.’ *The Japanese Journal of Pharmacology*, *40*(4), pp.551-559.

Tantisuwat, A. and Thaveeratitham, P., 2014 ‘Effects of smoking on chest expansion, lung function, and respiratory muscle strength of youths.’ *Journal of physical therapy science*, *26*(2), pp.167-170.

Teo, K.K., Ounpuu, S., Hawken, S., Pandey, M.R., Valentin, V., Hunt, D., Diaz, R., Rashed, W., Freeman, R., Jiang, L. and Zhang, X., 2006 ‘Tobacco use and risk of myocardial infarction in 52 countries in the INTERHEART study: a case-control study.’ *The lancet*, *368*(9536), pp.647-658.

Thijssen, D.H., Bruno, R.M., van Mil, A.C., Holder, S.M., Faita, F., Greyling, A., Zock, P.L., Taddei, S., Deanfield, J.E., Luscher, T. and Green, D.J., 2019 ‘Expert consensus and evidence-based recommendations for the assessment of flow-mediated dilation in humans.’ *European heart journal*, *40*(30), pp.2534-2547.

Tian, T., Jiang, X., Qin, R., Ding, Y., Yu, C., Xu, X. and Song, C., 2023 ‘Effect of Smoking on Lung Function Decline in a Retrospective Study of a Health Examination Population in Chinese Males.’ *Frontiers in Medicine*, *9*, p.843162.

Toda, N. and Okamura, T., 2003 ‘The pharmacology of nitric oxide in the peripheral nervous system of blood vessels.’ *Pharmacological reviews*, *55*(2), pp.271-324.

Toda, N. and Toda, H., 2010 ‘Nitric oxide-mediated blood flow regulation as affected by smoking and nicotine.’ *European journal of pharmacology*, *649*(1-3), pp.1-13.

United States. Public Health Service. Office of the Surgeon General, 2010 ‘*How tobacco smoke causes disease: the biology and behavioral basis for smoking-attributable disease: a report of the Surgeon General*.’ US Department of Health and Human Services, Public Health Service, Office of the Surgeon General.

Unverdorben, M., van der Bijl, A., Potgieter, L., Venter, C., Munjal, S., Liang, Q., Meyer, B. and Röthig, H.J., 2008 'Effects of different levels of cigarette smoke exposure on prognostic heart rate and Rate—Pressure-product parameters.' *Journal of cardiovascular pharmacology and therapeutics*, *13*(3), pp.175-182.

Van Ganse, W.F., Ferris Jr, B.G. and Cotes, J.E., 1972 'Cigarette smoking and pulmonary diffusing capacity (transfer factor).' *American Review of Respiratory Disease*, *105*(1), pp.30-41.

Vardavas, C.I., Anagnostopoulos, N., Kougias, M., Evangelopoulou, V., Connolly, G.N. and Behrakis, P.K., 2012 'Short-term pulmonary effects of using an electronic cigarette: impact on respiratory flow resistance, impedance, and exhaled nitric oxide.' *Chest*, *141*(6), pp.1400-1406.

Villarreal, F.J., Hong, D. and Omens, J., 1999 'Nicotine-modified postinfarction left ventricular remodeling.' *American Journal of Physiology-Heart and Circulatory Physiology*, *276*(3), pp.H1103-H1106.

Vlachopoulos, C., Aznaouridis, K. and Stefanadis, C., 2010 'Prediction of cardiovascular events and all-cause mortality with arterial stiffness: a systematic review and meta-analysis.' *Journal of the American College of Cardiology*, *55*(13), pp.1318-1327.

Vlachopoulos, C., Ioakeimidis, N., Abdelrasoul, M., Terentes-Printzios, D., Georgakopoulos, C., Pietri, P., Stefanadis, C. and Tousoulis, D., 2016 'Electronic cigarette smoking increases aortic stiffness and blood pressure in young smokers.' *Journal of the American College of Cardiology*, *67*(23), pp.2802-2803.

Vlachopoulos, C., Kosmopoulou, F., Panagiotakos, D., Ioakeimidis, N., Alexopoulos, N., Pitsavos, C. and Stefanadis, C., 2004 'Smoking and caffeine have a synergistic detrimental effect on aortic stiffness and wave reflections.' *Journal of the American College of Cardiology*, *44*(9), pp.1911-1917.

Vlachopoulos, C., Xaplanteris, P., Aboyans, V., Brodmann, M., Cífková, R., Cosentino, F., De Carlo, M., Gallino, A., Landmesser, U., Laurent, S. and Lekakis, J., 2015 'The role of vascular biomarkers for primary and secondary prevention. A position paper from the European Society of Cardiology Working Group on peripheral circulation: Endorsed by the Association for Research into Arterial Structure and Physiology (ARTERY) Society.' *Atherosclerosis*, *241*(2), pp.507-532.

Ward, S.A., 2021 'Ventilation/carbon dioxide output relationships during exercise in health.' *European Respiratory Review*, *30*(160).

Wasserman, K., 1984 'The anaerobic threshold measurement to evaluate exercise performance.' *American review of respiratory disease*, *129*(2P2), pp.S35-S40.

Wright, J.L., Hobson, J., Wiggs, B.R., Pare, P.D. and Hogg, J.C., 1987 'Effect of cigarette smoking on structure of the small airways.' *Lung*, *165*, pp.91-100.

Wu, J. and Sin, D.D., 2011 'Improved patient outcome with smoking cessation: when is it too late?.' *International journal of chronic obstructive pulmonary disease*, *6*, p.259.

Yano, T., Yunoki, T., Matsuura, R. and Arimitsu, T., 2009 'Relationship between hyperventilation and excessive CO2 output during recovery from repeated cycling sprints.' *Physiological research*, *58*, pp.529-535.

Yan, X.S. and D'Ruiz, C., 2015 'Effects of using electronic cigarettes on nicotine delivery and cardiovascular function in comparison with regular cigarettes.' *Regulatory Toxicology and Pharmacology*, *71*(1), pp.24-34.

Yuan, J.J., Li, H.M., Huang, Y., Han, X.R., Gao, Y.H., Zhong, N.S., Martinez-Garcia, M.A. and Guan, W.J., 2020 'Decreased ventilatory efficiency during incremental exercise in bronchiectasis.' *Journal of Thoracic Disease*, *12*(5), p.2717.

Zamel, N., Altose, M.D. and Speir, W.A., 1983 'Statement on Spirometiy: A Report of the

Section on Respiratory Pathophysiology of the American College of Chest Physicians.' *Journal of Asthma*, *20*(4), pp.307-311.

Zhu, S.H., Sun, J.Y., Bonnevie, E., Cummins, S.E., Gamst, A., Yin, L. and Lee, M., 2014 'Four hundred and sixty brands of e-cigarettes and counting: implications for product regulation.' *Tobacco control*, *23*(suppl 3), pp.iii3-iii9.

Zweerink, A., van der Lingen, A.L.C., Handoko, M.L., van Rossum, A.C. and Allaart, C.P., 2018 'Chronotropic incompetence in chronic heart failure: a state-of-the-art review.' *Circulation: Heart Failure*, *11*(8), p.e004969.