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Reduced skeletal muscle endurance and ventilatory efficiency during exercise in adult smokers without airflow obstruction

Ahmed S. Sadaka[1,2], Azmy Faisal[1,3,4], Yehia M. Khalil[2], Sahar M. Mourad[2], Mohamed H. Zidan[2], Michael I. Polkey[1,5], Nicholas S. Hopkinson[1]

[1]*National Heart and Lung Institute, Imperial College London, London, UK*

[2]*Alexandria University, Faculty of Medicine, Chest Department, Alexandria, Egypt*

[3]*Department of Sport and Exercise Sciences, Manchester Metropolitan University, Manchester, UK*

[4]*Alexandria University, Faculty of Physical Education for Men, Alexandria, Egypt*

[5]*Respiratory Medicine, Royal Brompton and Harefield NHS Foundation Trust, London UK,*

Short Title: Muscle and Exercise Performance in Smokers

Correspondence: Ahmed S. Sadaka

NHLI, Imperial College London

Respiratory Muscle Laboratory

Royal Brompton Hospital Campus, Fulham Rd, London, UK – SW3 6NP

Phone +20 100 5433 152

Email ahmad.sadaka@alexmed.edu.eg

ABSTRACT (Word count: 249)

Background: Smokers without airflow obstruction have reduced exercise capacity, but the underlying physiological mechanisms are not fully understood.

Aim: To compare quadriceps function assessed using non-volitional measures, and ventilatory requirements during exercise, between smokers without airway obstruction and never-smoker controls.

Study Design and Methods: Adult smokers (n=20) and never-smoker controls (n=16) aged 25-50 years with normal spirometry, underwent incremental cycle cardiopulmonary exercise testing to exhaustion with measurement of symptoms and dynamic lung volumes. Quadriceps strength and endurance were assessed non-volitionally using single and repetitive magnetic stimulation. Quadriceps bulk was assessed using ultrasound, as rectus-femoris cross-sectional area (Q_{RF}-CSA). Physical activity level was quantified using the SenseWear^{TM} armband worn for 5 days.

Results: Smokers had lower peak exercise workload, peak oxygen consumption and anaerobic threshold (AT) compared to controls (170±46 vs. 256±57 W; 2.20 ±0.56 vs. 3.18 ±0.72 L/min; 1.38±0.33 vs. 2.09±0.7 L/min, respectively; $p<0.01$ for all). Quadriceps endurance was lower in smokers (Δ force-time integral 54.9±14.7% vs. 40.4±14.7%; $p=0.007$), but physical activity, quadriceps strength and bulk were similar between groups. Smokers displayed higher ventilation (120W: 52.6±11.8 vs. 40.7±6.0 L/min; $p<0.001$), decreased ventilatory efficiency (higher $\dot{V}_E/\dot{V}CO_2$) and were more breathless with greater leg fatigue at iso-workloads and iso-ventilation levels compared to never-

smoker controls. Smokers showed no mechanical constraints on tidal volume expansion during exercise or ventilatory limitation at peak exercise.

Conclusion: Adult smokers without airflow obstruction have reduced skeletal muscle endurance and ventilatory efficiency compared to never-smoker controls, despite similar daily physical activity levels, which contributed to reduced peak exercise capacity.

Keywords: smoking, exercise, skeletal muscle, magnetic stimulation, ventilatory efficiency

New & Noteworthy:

In adult smokers without airflow obstruction, the contributions of pulmonary and skeletal muscle functions to reduced exercise capacity are unclear. We found non-COPD smokers had decreased exercise capacity and muscle endurance though strength was preserved compared to never-smoking controls. Exercise endurance was associated with quadriceps endurance and CO transfer factor. Despite similar physical activity levels, smokers developed leg fatigue, breathlessness and displayed increased ventilation with reduced ventilatory efficiency at lower workloads, without exhibiting ventilatory constraint.

INTRODUCTION

Smoking is an etiological factor in several diseases which ultimately cause reduced exercise capacity (12). In particular, smokers are at increased risk of developing chronic obstructive pulmonary disease (COPD) (9, 43, 44). However, reduced physical performance in apparently healthy smokers in the absence of airflow obstruction has also been reported, with studies suggesting that impairments in cardiac (19), lung (15, 44), vascular (4, 17, 19), and skeletal muscle (36, 56), function may contribute to this. Nonetheless, the exact pathophysiological mechanisms responsible for exercise limitation in smokers without COPD remain unclear and poorly understood.

Chronic exposure to tobacco smoke has been shown to have a negative impact on skeletal muscle structure and function in adult smokers without airflow obstruction (26, 34, 36, 56). Orlander et al. were among the first to describe the functional and ultrastructural changes occurring in the muscles of smokers (38). Biopsies from the vastus lateralis showed a reduced proportion and cross-sectional area of the oxidative Type I, fatigue resistant fibers, and an increased proportion of glycolytic Type IIb fibers in smokers, although of note the participants also had low physical activity (38). This was associated with a reduction in muscle oxidative capacity (38), increased lactate production during exercise (11), and reduced skeletal muscle fatigue resistance (36, 57). Chronic exposure to tobacco smoke has been associated with ubiquitin-mediated proteolysis (41, 46). Reduced oxidative capacity and fatigue resistance in smokers were linked to impaired oxygen delivery due to the formation of carboxyhaemoglobin (HbCO) (35), as well as inhibition of intracellular oxygen transport by combination of CO with myoglobin, and oxidative phosphorylation by blockage of cytochrome oxidase, that can be

88 further hampered by cyanide in cigarette smoke (31, 45). Smoking-related skeletal muscle
89 dysfunction was shown to be associated with a lower peak oxygen uptake (V̇O2) (31, 45).

90 It has previously been shown that older smokers without COPD are more breathless during
91 exercise. This was associated with lower lung diffusion capacity (DL_{CO}) (25), and increased
92 airway resistance, diaphragmatic effort and inspiratory neural drive but no difference in
93 exercise ventilatory demand (15). Additionally, COPD smokers have been shown to have lower
94 exercise capacity and ventilatory efficiency compared to matched COPD non-smokers (50).
95 Evidence of lower ventilatory efficiency (higher $\dot{V}_E/\dot{V}CO_2$) among smokers without COPD has
96 been contradictory (15, 53).

97 We therefore undertook a study in young and middle-aged adults to reduce the potential
98 confounding effect of ageing on respiratory and skeletal muscle function. We aimed to compare
99 ventilatory requirements during exercise and quadriceps function assessed using non-volitional
100 techniques between smokers without airflow obstruction and never-smokers. We hypothesized
101 that skeletal muscle endurance would be impaired even in ostensibly "healthy" adult smokers,
102 and that this together with ventilatory inefficiency would be key determinants of reduced
103 exercise capacity.

104

105 **METHODS**

106 *Subjects*

107 We recruited recreationally active adult smokers aged between 25 and 50 years without airflow
108 obstruction – forced expiratory volume in 1 second (FEV_1) / forced vital capacity (FVC) > 70%
109 and above the lower limit of normal and FEV_1 >80% predicted (43). Exclusion criteria included:

any drug or medical condition that could affect muscle function (e.g. congestive heart failure, thyroid dysfunction, arthritis or muscle disease), contraindication to magnetic nerve stimulation (e.g. permanent pacemaker), any chronic respiratory disease or a body mass index <18.5 or >35 kg/m^2. Sixteen recreationally active healthy never-smoking controls in the same age range were recruited for comparison. Both groups were recreationally exercising 2 to 3 times weekly. The study was approved by the UCLH Alpha Research Ethics Committee (reference number-09/H0715/37) and all participants gave written informed consent.

Study Procedures

The study was cross-sectional and involved two visits; visit 1: smoking history and current status confirmed by exhaled carbon monoxide level (23). Detailed pulmonary function tests (PFT), including spirometry, DL_{CO} and body plethysmography, were performed using CompactLab System (Jaeger, CareFusion®, Heidelberg, Germany) according to the ATS/ERS standards (28, 32, 54). Participants were instructed to refrain from smoking for at least 1 hour before PFT.

Following 3 minutes of rest and 1-minute warm-up of unloaded cycling, a stepwise incremental cardiopulmonary exercise (CPET) was performed at 20W/min on an electronically braked cycle ergometer (Ergoselect 100, Ergoline, Bitz, Germany). Measurements included: standard breath-by-breath cardiopulmonary variables; breathing pattern parameters (MasterScreen™ CPX, Viasys Healthcare GmbH, Hoechberg, Germany); heart rate monitoring by 12-lead electrocardiography and oxygen saturation by pulse oximetry (SpO_2) (3). Arterialized blood gases were measured at rest and peak exercise. Anaerobic threshold (AT) was estimated from the break point in the curve of $\dot{V}CO_2$ as a function of $\dot{V}O_2$ (V-slope method) (6) and confirmed by the point at which $\dot{V}E$-to-$\dot{V}O_2$ ratio increased without an increase in $\dot{V}E$-to-$\dot{V}CO_2$ ratio.

133 Operating lung volumes were derived from inspiratory capacity (IC) maneuvers at rest, and
134 during exercise. Leg and breathing discomfort were assessed using the modified 10-unit Borg
135 scale (7).
136 A multi-sensor accelerometer device (SenseWear® Armband) (49) was used to measure daily
137 step count, basal metabolic rate and physical activity levels (PAL) over 5 successive days
138 including 2 weekend days to account for any difference in physical activity (47). PAL was
139 calculated by dividing the total daily energy expenditure by the basal metabolic rate (derived
140 from measured resting energy expenditure) (22). A daily wearing time ≥22.5 hours/day was
141 required for a measurement day to be valid (55).
142 At the second visit, a week later, the physical activity monitors were collected, and skeletal
143 muscle parameters were assessed.
144 *Quadriceps ultrasound*
145 Quadriceps rectus femoris cross-sectional surface area (Q_{RF}-CSA) of the dominant leg was
146 measured at 60% of the distance between the anterior superior iliac spine and the superior
147 patellar border by B-mode ultrasonography using a 4 MHz curvilinear probe (Siemens Acuson®
148 S2000) in a rested supine position as described previously (49). After applying a generous
149 amount of gel, the transducer was placed perpendicular to the Q_{RF} long axis with the least
150 pressure to avoid image distortion. One operator, AS, performed all ultrasound scans to avoid
151 inter-observer variability. Post-image-acquisition analysis and planimetric tracing was done to
152 measure cross-sectional surface area (Q_{RF}-CSA) and calculate the contractility index (% change
153 in CSA between contracted and relaxed states), using ImageJ® software version 1.51n. The
154 average reading for Q_{RF}-CSA of at least 3 images within 5% was used.
155

156

157 *Quadriceps power and endurance*

158 Subjects performed at least 5 quadriceps maximum voluntary contractions (QMVC) as
159 described previously (13). A QMVC trial was considered valid if the participant maintained the
160 force achieved for at least 1 second. Trials that did not meet this validation criteria were
161 excluded from the analysis and the highest value recorded of the validated maximal
162 contractions was taken as QMVC. Magnetic femoral nerve stimulation protocol was used to
163 elicit unpotentiated quadriceps twitches (Qtw) using a Magstim® mono-pulse 200 unit (42). An
164 interpolated twitch was used to ascertain the maximality of effort during the QMVC maneuver
165 (42).

166 Quadriceps endurance was assessed non-volitionally with a flexible mat coil wrapped around
167 the quadriceps to deliver 50 trains of magnetic repetitive muscle stimulations (rMS) at a
168 frequency of 30 Hz, 0.4 duty cycle and a power adjusted to yield approximately 30% of QMVC
169 force. The decay in force was used to calculate the time needed to reach 70% of the initial
170 force-time integral (T_{70}) and the percentage decline in force-time integral (Δ-FTI) (51).

171 *Statistical analysis*

172 A sample size of 16 subjects per group was estimated to detect differences in non-volitional
173 quadriceps fatigability and exercise capacity (effect sizes of 0.93 & 1.89 respectively) between
174 the two study groups using a 2-sample t-test with a power of 80% and a two-tailed alpha level
175 of 0.05 (15, 36). Previously, similar difference in muscle endurance was apparent in groups of 8
176 patients with COPD and healthy matched controls (T_{70}: 55.6±26 vs. 121±38.7sec, $p < 0.05$) (51).

Results are presented as mean±SD. Unpaired two-tailed t-test was used to compare between group differences. Spearman's rank correlation analysis was used to estimate the strength of associations between pulmonary and muscular functional parameters. A hierarchical multivariable linear regression analysis was performed to identify which factors were most strongly associated with exercise performance. Independent variables predicting peak oxygen consumption ($\dot{V}O_2$, ml/min/kg) were selected for the multivariable regression model inclusion based on clinical relevance and prior univariate analysis with a p-value <0.2. Assumptions for linear regression analysis were all tested. A p-value of <0.05 was considered statistically significant. IBM SPSS version 22 Windows statistical package was used for analysis.

Results

The smoker and never-smoker groups were well-matched for age, height, body mass, body mass index and gender (Table 1). Physical activity level did not differ between smokers and never-smoker controls (1.53±0.2 vs 1.48 ±0.3; p=0.596, 95% CI = -0.14 – 0.24) nor did mean daily step count (10,254 ±3,838 vs. 8,854 ±3,025 step/day; p=0.289).

Skeletal Muscle Parameters

Quadriceps strength was not statistically different comparing smokers and never-smoker controls; QMVC 38.8±10.0 kg vs. 45.2±14 kg (p=0.121; 95% CI for difference -1.8 to 14.5), Qtw 13.3±3.3 kg vs.15.6±4.3 kg (p=0.078, 95% CI= -0.3 to 4.9) respectively, (Fig.1a,b; supplementary S1–https://doi.org/10.6084/m9.figshare.13378865). Twitch interpolation showed similar degrees of voluntary muscle activation (89.2±12.5% vs 91.4±9.0%, p= 0.429). By contrast, quadriceps endurance was significantly reduced, with a more rapid decline in force elicited by

199 rMS in smokers; T_{70} 85.8±26.6 vs. 133.8±68.3 seconds (p=0.016, 95% CI= 10.0 to 85.9) (Fig.2)
200 and Δ-FTI 54.9±14.7% vs. 40.4±14.7% (p=0.007, 95% CI= -24.7 to -4.3). Δ-FTI was significantly
201 associated with peak $\dot{V}O_2$ (r= -0.371, p=0.028) and peak work rate (r= -0.366, p=0.031).
202
203 Ultrasound imaging showed a numerically smaller resting Q_{RF}-CSA in smokers though this was
204 not statistically different from never-smoker controls (740.4±212.3 vs. 871.8±191.1 mm^2;
205 p=0.072, 95% CI= -12.3 – 275.1). The contractility index was similar between smokers and
206 never-smoker controls (16.4±24.4 vs 8.2±8.0%; p=0.218, 95% CI = -21.4 – 51.0, respectively).
207
208 *Lung function and cardiopulmonary exercise parameters*
209 Table 1 shows PFT for both groups. The only parameter with that differed significantly was
210 DL_{CO} which was lower in smokers (80.8 ± 12 vs. 91.5 ±10.9% predicted; p=0.01; 95% CI= 2.7-
211 18.4).
212 Smokers achieved a lower peak $\dot{V}O_2$ and peak $\dot{V}_E$ compared to never-smoker controls (Table 2).
213 AT occurred at a significantly lower absolute and relative to maximum workloads in smokers
214 compared to never-smoker controls (83.5±26.7 vs. 153.8±52.5W; 50.3±9.4 vs. 59.1±11.6%,
215 p<0.05 for both). Also, AT occurred at a significantly lower $\dot{V}O_2$ in smokers (1.38 ±0.33 vs
216 2.09±0.7 L/min; p=0.001; 95% CI= 0.31-1.12). However, this corresponded to a similar
217 percentage of the peak $\dot{V}O_2$ (63.8 ±8.2 vs. 65.3 ±12.2%, p=0.931).
218
219 Ventilatory requirements and ventilatory equivalent for CO_2 ($\dot{V}_E/\dot{V}CO_2$) were higher in smokers
220 compared to healthy controls at iso-workloads 80-120W (Fig. 3 a, b). Additionally, nadir values
221 for $\dot{V}_E/\dot{V}CO_2$ were higher in smokers (25.4 ±2.6 vs. 22.8 ±2.2; p= 0.005, 95% CI= -4.3 – 0.8). Tidal

volume was significantly smaller and breathing frequency was significantly higher in smokers vs. controls at submaximal ventilation (Fig.4 a, b), and tidal volume ventilatory inflection points tended to occur at a lower ventilatory level in smokers (58.0 ±18.8 vs. 71.3±27.8 L/min, p = 0.098). There were no differences in IC, IRV (Fig.4 c, d), operating lung volumes or dead-space values between smokers and control groups (supplementary figure S1– https://doi.org/10.6084/m9.figshare.13378865). Heart rate was significantly higher and O_2 pulse (a surrogate marker for stroke volume) was significantly lower in smokers throughout submaximal exercise workloads (supplementary figure S2– https://doi.org/10.6084/m9.figshare.13378865). DL_{CO} showed a positive correlation with peak $\dot{V}O_2$ (r=0.54, p=0.001) and AT (r=0.52, p=0.002) and was negatively associated with nadir $\dot{V}_E/\dot{V}CO2$ (r= -0.38, p= 0.03). Borg scores for leg fatigue and dyspnea were significantly higher in smokers at iso-workloads (Fig. 5a, b) and iso-ventilation levels (Fig. 5c, d).

Analysis of symptoms reported identified dyspnea, leg fatigue or a combination of both to be the main symptom limiting exercise in 10%, 45% and 45% of smokers and 13%, 31% and 56% of non-smoker controls respectively. Although leg fatigue was more frequently reported as the dominant symptom at the termination of exercise among smokers, this was not found to be statistically significant compared to never-smoker controls (45 vs. 31%, p= 0.720).

The multivariable linear regression analysis assumptions were met with residuals showing normal distribution and homoscedasticity. Variance inflation factor values as well as correlation coefficients confirmed absence of multicollinearity. Age, DL_{CO}% predicted and T_{70} were found to be independent predictors of peak exercise oxygen consumption with the regression model predicting 60.2% of the variance with an adjusted R^2 of 0.602, p<0.0005 (Table 3).

DISCUSSION

The main finding of this study were that exercise capacity and peak $\dot{V}O_2$ was reduced in smokers even in the absence of airflow obstruction and that this was accompanied by increased breathlessness and reduced ventilatory efficiency and associated with CO diffusing capacity, though there was no evidence of mechanical constraint on ventilation. In addition, exercise capacity limitation in smokers was associated with a marked reduction in quadriceps endurance assessed using non-volitional techniques and smokers demonstrated a lower anaerobic threshold and developed symptoms of muscle fatigue at lower workloads. Importantly, daily physical activity did not differ between groups, suggesting that these findings cannot be explained by deconditioning and are likely to represent a direct effect of smoking on skeletal muscle.

Significance of the findings

Quadriceps endurance was clearly reduced in smokers. Consistent with our findings, it has been demonstrated in quadriceps biopsies that smokers have a fiber type shift from the slow twitch type I, oxidative fatigue resistant fibers, to the fast twitch type II glycolytic muscle fibers (34, 38). Another potential mechanism is that an increase in systemic inflammatory mediators in smokers, for example interleukin-6 and protein synthesis inhibition, could promote a secondary sarcopenia phenotype (40, 41, 52). However, against the latter are observations from patients with established COPD where skeletal muscle abnormalities are common (20, 37, 49) but systemic inflammation does not seem to be a key factor (33).

Carbon monoxide and reactive oxygen species produced in cigarette smoke, together with endothelial dysfunction and increased vascular resistance, negatively impact oxygen extraction

kinetics and impair mitochondrial oxidative capacity leading to impaired muscle function (1, 16, 24, 25, 34, 41), with increased lactate production during exercise (11) consistent with our finding of lower AT and O2 pulse in the smoking group. Additionally, Klotho, an anti-ageing hormone and transmembrane protein, has been found to be negatively expressed in muscles of current smokers (40).

Our finding of similar physical activity levels in both groups argues against deconditioning being the primary cause of the functional difference. Arising from this, although both skeletal muscle abnormalities and physical activity limitation are present even in mild to moderate COPD (10, 34, 49), we should not assume that the presence of the latter completely explains the former. Muscle oxidative capacity has been shown to be decreased in both lower and upper limbs in COPD patients compared to non-COPD smokers; while physical activity was not statistically different (2). This echoes with our results of similar physical activity but evident differences in muscle endurance and exercise capacity between smokers and never-smoker controls. PAL and daily step counts are indicative of physical activity, but this is dependent on behavioral factors and may not, as observed here, reflect maximum exercise capacity.

The reduced muscle endurance in our smoking cohort was an important contributor to limited exercise capacity and was also reflected in higher Borg scores of leg fatigue among smokers at equivalent work-rates. The lower anaerobic threshold among smokers corresponded to a similar percentage of the peak $\dot{V}O_2$, however it should be noted that all the subjects were considered healthy without any evidence of disease. Higher levels of breathlessness and leg fatigue were also demonstrated at iso-ventilatory levels as shown in fig. 5c,d. Build-up of lactate along with afferent stimuli from peripheral mechanoreceptors would also have contributed to

increased ventilatory drive, increased minute ventilation and the sensation of dyspnea during physical activity (39). Smokers did not demonstrate ventilatory limitation compared to never-smokers, as evidenced by similar dynamic lung volume changes (supplementary figure S1-https://doi.org/10.6084/m9.figshare.13378865) and crucially a substantially greater ventilatory reserve in smokers at peak exercise (Fig.3). The higher heart rate at iso-work rates also supports a role for muscle dysfunction, and likely allows less filling time and lower stroke volume as shown by lower O_2 pulse values (supplementary figure S2-https://doi.org/10.6084/m9.figshare.13378865). The relative reduction in cardiac output will have impacted intra-muscular oxygen delivery kinetics.

Ultrasound measurements of the rectus femoris were not found to be statistically different in both groups of participants suggesting similar muscle bulk and consistent with the lack of difference we observed in measures of muscle strength; however for both strength and bulk in never smokers the data admit the possibility that a larger study might have disclosed statistically significant differences between groups. This would not detract from the current observation that the greatest impairment was seen in endurance. Of note, although muscle weakness has been demonstrated in early COPD compared to healthy controls (49), Gagnon et al. found no difference in strength, measured using QMVC and potentiated quadriceps twitch force, between non-COPD smokers and GOLD stage 1 COPD patients (16).

Despite normal spirometry, lung diffusion capacity was lower in the smoking group. This has been previously shown to be associated with higher dyspnea scores and worse exercise tolerance in smokers with and without airflow limitation (25) and may represent pulmonary

microvascular loss. Impaired gas exchange in smokers might have impacted exercise capacity (21). Although end-exercise arterial blood gas values did not differ between groups, continuous measurements were not performed to investigate the possibility of gas exchange abnormalities at submaximal exercise as demonstrated in mild COPD (14). During incremental exercise, smokers had higher ventilation and breathing rates at iso-work rates. This contrasts with previous findings in elderly smokers without COPD which showed similar ventilatory requirements at submaximal work-rates compared to healthy controls (15). There was no significant divergence in operating lung volumes nor was the tidal volume ventilatory inflection point different between groups suggesting that mechanical ventilatory limitation was not a determining factor in exercise impairment. This has also been the case in older smokers (15) but contrasts to findings in early COPD (14). The increased ventilatory response we found was also accompanied by a higher nadir $\dot{V}_E/\dot{V}CO_2$. Although this could suggest an increased physiological dead space, we found no difference between groups in the V_D/V_T ratio. The increase in $\dot{V}_E/\dot{V}CO_2$ found in smokers could be due to a number of non-exclusive possible mechanisms; a) the pulmonary endothelial dysfunction caused by smoke induced down-regulation of endothelial nitric oxide synthase and early onset emphysema especially given the lower observed diffusion capacity (4, 18), b) a heightened ventilatory drive secondary to nicotine-induced sympathetic stimulation, c) earlier lactic acidosis (as shown by a lower AT) and increased ergo-receptor activity from glycolytic lower endurance muscles fibers (39); this latter possibility would of course also be consistent with the demonstrated reduction in muscle endurance. In our cohort, there was a correlation between ventilatory inefficiency (high $\dot{V}_E/\dot{V}CO_2$) and low DL_{CO}% predicted (r=-0.379, p=0.03). Different groups have related lower diffusion capacity in smokers to peripheral endothelial dysfunction and decreased flow-

mediated dilatation secondary to the oxidative and pro-inflammatory environment created by smoke exposure (17, 53). Similar multisystemic findings have been described in mild COPD which suggests changes in muscle function and pulmonary dynamics during exercise could start early on, with or without symptoms, before evident airflow limitation (10, 14, 15).

Critique of the Methods

We focused on quadriceps function as this is an important muscle used for locomotion (36, 56) and studied relatively young participants to ascertain the impact smoking has prior to a significant impact of ageing on muscle or respiratory function. Importantly, physical activity, which is an important mediator of exercise capacity, was quantified objectively using an activity monitor. It is notable that despite lack of significant respiratory symptoms and a relatively low smoking history, with a mean of 14 pack-years, we could still show significant differences in muscle fatigability and exercise performance. Importantly, previously validated non-volitional magnetic stimulation techniques were used to test muscle strength and endurance (42, 51) which are not dependent on participant motivation or coordination.

Previous studies have shown an association between smoking and muscle strength, however physical activity was either not measured or not accounted for. In addition, current versus past smoking history was often not differentiated; an important factor to be considered given the unclear temporal effects of smoking on muscle function and structure (5, 38). In the present study we overcame this by measuring physical activity and enrolling only never-smokers as controls.

Our results contrast those of Boyer et al (8) who found no difference in muscle function between smokers and non-smokers. However, there are several important differences between our study design and theirs. First, they measured hand-grip strength rather than quadriceps strength and it is known that upper limb strength may remain normal even in smokers and people with COPD despite the presence of quadriceps weakness (30). In addition, they used a test of maximal voluntary contraction rather than a non-volitional test so motivation or coordination may have played a role. In our group, different measures of quadriceps muscle power failed to unveil a difference, in agreement with previous findings by Morse et al. who showed similar muscle strength and voluntary activation levels in young male smokers (36). We opted to analyze quadriceps strength using absolute and bodyweight-corrected values (QMVC/Body weight). In addition, adjustment for muscle cross-section in the contracted state (QMVC/RF-CSA-c) was tested.

As they arise from an observational study, the data cannot confirm a causative relation between smoking and the aforementioned variables. Demonstration of an improvement in parameters of muscle function and ventilatory efficiency during exercise after successful smoking cessation would help to support a causal association. Despite not performing a more thorough evaluation of small airways function using impulse oscillometry or nitrogen washout; normal residual volume & residual volume/total lung capacity ratios together with absence of evidence of dynamic hyperinflation argue against this being an important factor affecting exercise performance. Assessment of muscle mass would have been optimal with the use of dual energy X-ray absorptiometry or magnetic resonance imaging; the latter might also have confirmed anaerobic metabolism during contraction, or the presence of increased muscle adiposity as has been noted in COPD (29, 48). No muscle biopsies were taken in this study, so we can only

speculate as to the underlying pathophysiological mechanisms in smokers' muscles, although we previously showed that reduced endurance was associated with reduced type I myosin heavy chain percentage in patients with COPD (51). Lastly, although the use of maximal cardiopulmonary exercise testing allowed accurate measurement of ventilatory and metabolic variables along with assessment of changes in lung volumes and pulmonary mechanics in smokers and control subjects, an additional endurance constant workload exercise test would have allowed an additional perspective on exercise performance and association between cycle endurance time and quadriceps muscle endurance.

Conclusion

These data provide clear evidence of exercise limitation and skeletal muscle impairment in smokers without airflow obstruction. These changes may be precursors to the subsequent development of the multi-systemic effects documented in people with COPD. Knowledge of this should stimulate further research into strategies to prevent or reverse muscle impairment in individuals who are or have been smokers. In addition, knowledge that smoking impairs physical performance may provide a further argument or incentive for smokers to try to quit, particularly within younger peer groups (27) where physical performance may be more immediately salient than the risk of future ill health.

Abbreviations

COPD	Chronic obstructive pulmonary disease
$\dot{V}O2$	Oxygen uptake
AT	Anaerobic threshold
DL_{CO}	Lung diffusing capacity for carbon monoxide
$\dot{V}_E/\dot{V}CO_2$	Ventilatory equivalent for carbon dioxide
PFT	Pulmonary function test
FEV1	Forced expiratory volume in 1 second
FVC	Forced vital capacity
SpO2	Oxygen saturation by pulse oximetry
IC	Inspiratory capacity
QRF-CSA	Quadriceps rectus femoris cross-sectional surface area
QMVC	Quadriceps maximum voluntary contraction
Qtw	Quadriceps twitch force
rMS	Repetitive muscle stimulation
T_{70}	Time to 70% of the initial force-time integral
Δ-FTI	Percentage decline in the force-time integral

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Authors' contribution: ASS and AF are joint first authors. ASS and AF contributed to recruitment, data collection, data analysis and writing of the initial draft. YMK, SMM and MHZ contributed to the interpretation of the results and critically revising the manuscript draft. NHS and MIP contributed to the conception of the study, data interpretation, and writing the manuscript.

All authors approved the final version of the manuscript to be submitted for publication and agree to be accountable for all aspects of the research work presented.

Table Footnotes

Table 1: Subject characteristics

Definition of abbreviations: BMI= body mass index; PAL= physical activity level; MRC= medical research council; FEV_1= forced expiratory volume in the first second; FVC= forced vital capacity; TLC= total lung capacity; RV= residual volume; FRC= forced residual capacity, DL_{CO}= lung diffusing capacity for carbon monoxide; K_{CO}= carbon monoxide diffusion coefficient; P_aO_2= arterial oxygen partial pressure; P_aCO_2= arterial carbon dioxide partial pressure.
All values are expressed as mean±SD unless otherwise stated.

Table 2: Peak cardiopulmonary exercise parameters

Definition of abbreviations: $\dot{V}O_2$= oxygen consumption; $\dot{V}_E$= minute ventilation; MVV= maximum voluntary ventilation; $\dot{V}_E/\dot{V}CO_2$= ventilatory equivalent for carbon dioxide; S_pO_2= oxygen saturation measured by pulse oximetry; RR= respiratory rate; V_T= tidal volume; IC= inspiratory capacity; IRV=inspiratory reserve volume; RER= respiratory exchange ratio; P_aO_2= arterial oxygen tension; P_aCO_2= arterial carbon dioxide tension; V_D/V_T= dead-space ratio; A-aDO_2= alveolar-arterial oxygen gradient; Δ A-aDO_2= change in alveolar-arterial oxygen gradient from rest to peak exercise.
All values are expressed as mean±SD unless otherwise stated.

Table 3: Multivariable linear regression model for $\dot{V}O_2$ (ml/min/kg) prediction

Definition of abbreviations: $\dot{V}O_2$= oxygen consumption; DL_{CO}% pred.= lung diffusing capacity for carbon monoxide as a percentage of predicted; T_{70} = Time to 70% of the initial force-time integral.
Adjusted R^2 = 0.602, $p<0.0005$

Figure Legends

Figure 1: Box and whiskers plot showing quadriceps force in smokers without COPD and healthy controls in terms of a) quadriceps maximum voluntary contraction and b) non-volitional quadriceps twitch force elicited by magnetic stimulation of the femoral nerve. No significant difference in force was found between both groups.

Figure 2: Quadriceps endurance curves showing the rate of decline in quadriceps force-time integral with repetitive magnetic muscle stimulation in smokers without COPD and healthy controls. Smokers show more rapid decline in force generation compared to healthy controls with significant differences in T_{70} and after 20, 30, 40 and 50 trains of stimulation indicative of lower endurance in smokers. Values are presented as mean±SEM. *p<0.05
T_{70}= time to reach 70% of initial force-time integral

Figure 3: Ventilatory responses to incremental cardiopulmonary exercise test in smokers without chronic obstructive pulmonary disease and healthy controls showing a) increased minute ventilation at iso-work rates and lower peak work rate in smokers with normal ventilatory reserve at peak exercise in both groups; b) decreased ventilatory efficiency in smokers (higher nadir $\dot{V}_E/\dot{V}CO_2$); c) lower end-tidal carbon dioxide values and d) lower oxygen saturation among smokers. Values are presented as mean±SEM. Triangles represent tidal volume ventilatory inflection point. Top horizontal dashed and solid lines in (a) indicate maximum voluntary ventilation levels for smokers and healthy controls respectively. Zero workload represents the unloaded warmup. *p<0.05.
$\dot{V}_E/\dot{V}CO_2$= ventilatory equivalent for carbon dioxide; $P_{et}CO_2$= partial pressure of end-tidal carbon dioxide; S_pO_2= oxygen saturation measured by pulse oximetry.

Figure 4: Breathing pattern (a,b) and respiratory mechanical (c,d) responses to incremental cardiopulmonary exercise test in smokers without chronic obstructive pulmonary disease and healthy controls. Values are presented as mean±SEM. Triangles represent tidal volume ventilatory inflection point. The shaded area in (d) represents the minimal IRV reached by both groups at the exercise peak. Zero workload represents the unloaded warmup. *p<0.05.

Figure 5: Perception of breathlessness and leg fatigue in smokers without chronic obstructive pulmonary disease and healthy controls versus workloads (a, b) and ventilation (c,d) during incremental cardiopulmonary exercise tests. Values are presented as mean±SEM. Triangles represent tidal volume ventilatory inflection point. Zero workload represents the unloaded warmup. *p<0.05

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Table 1*:* Participant characteristics

	Smokers (n=20)	**Never-Smoker Controls (n=16)**	**P Value**
Age, years	40.1 ± 6.3	38.7 ±7.2	0.454
Male: Female (%female)	14:6 (30)	11:5 (31)	0.677
Height, m	1.71 ±0.07	1.77 ±0.11	0.081
Weight, kg	74.5 ±12.4	78.8 ±16.3	0.370
BMI, kg/m^2	25.4 ±4.3	24.9 ±3.1	0.713
Current smokers, %	100	0	
Step count/day, Steps	10254 ±3838	8854 ±3025	0.289
PAL index	1.48 ±0.27	1.53 ±0.23	0.584
MRC dyspnea score	1.1 ±0.3	1.0 ±0.0	0.163
Resting Lung Functions			
FEV_1, L	3.56 ±0.72	3.92 ±0.70	0.137
FEV_1% pred.	99.8 ± 26.5	104.0 ±13.2	0.915
FVC, L	4.50 ±0.84	5.09 ±1.03	0.068
FVC% pred.	111.0 ±14.2	110.0 ±13.8	0.831
FEV_1/FVC, %	78.6 ±5.7	76.8 ±4.8	0.307
TLC, L	6.34 ±1.09	7.05 ±1.38	0.90
TLC% pred.	105.1 ±12.1	105.8 ±11.3	0.863
RV, L	1.88 ±0.49	1.99 ±0.48	0.526
RV%	106.8 ±2.1	106.0 ±19.2	0.907
RV/TLC, %	29.6 ±5.2	28.1 ±3.8	0.351
FRC, L	3.09 ±0.82	3.53 ±0.82	0.114
FRC% pred.	101.7 ±21.6	108.8 ±22.3	0.345
$DL_{CO,}$ mL/min/mmHg	8.22 ±1.7	9.92 ±2.1	0.011
DL_{CO} % pred.	80.8 ±12.0	91.5 ±10.9	0.01
K_{CO}, mL/min/mmHg/L	1.46 ±0.22	1.59 ±0.21	0.104
K_{CO}% pred.	81.8 ±14.2	91.2 ±12.1	0.044
P_aO_2, kPa	12.0 ± 1.1	12.7 ±1.3	0.142
$P_aCO_{2,}$ kPa	5.01 ±0.49	4.84 ±0.55	0.324

Definition of abbreviations: BMI= body mass index; PAL= physical activity level; MRC= medical research council; FEV_1= forced expiratory volume in the first second; FVC= forced vital capacity; TLC= total lung capacity; RV= residual volume; FRC= forced residual capacity, DL_{CO}= lung diffusing capacity for carbon monoxide; K_{CO}= carbon monoxide diffusion coefficient; P_aO_2= arterial oxygen partial pressure; P_aCO_2= arterial carbon dioxide partial pressure.

All values are expressed as mean±SD unless otherwise stated.

Table 2: Peak cardiopulmonary exercise parameters

	Smokers (n=20)	**Never-Smoker Controls (n=16)**	**P Value**
Work rate, watts	169.25 ±46.4	256.25 ±56.7	<0.0005
$\dot{V}O_2$, L/min	2.20 ±0.56	3.18 ±0.72	<0.0005
$\dot{V}O_2$, ml/min/kg	29.7±6.3	40.5±6.2	<0.0005
$\dot{V}_E$, L/min	78.4 ±16.9	123.4 ±41.7	<0.0005
$\dot{V}_E$/MVV, %	57.0 ±15.2	78.1 ±18.0	0.001
$\dot{V}_E/\dot{V}CO_2$	29.7 ±4.7	31.0 ±5.1	0.433
S_pO_2, %	96.5 ±2.9	97.5 ±2.0	0.258
HR, beat/min	160 ±12	174 ±12	0.001
O_2/HR, ml/beat	13.5 ±3.4	18.3 ±4.6	0.001
RR, breath/min	35 ±8	42 ±9	0.021
V_T, ml	2.31 ±0.62	2.98 ±0.78	0.007
IC, L	3.39 ±0.54	3.88 ±0.84	0.041
V_T/IC %	67.7 ±0.1%	76.7 ±0.1	0.023
Δ IC, L	0.17 ±0.42	0.28 ±0.74	0.549
IRV, L	1.32 ±0.67	1.61 ±1.04	0.320
RER	1.25 ±0.1	1.24 ±0.09	0.623
Dyspnea, Borg units	5.6 ±2.4	6.2 ±2.5	0.457
Leg fatigue, Borg units	6.3 ±2.3	6.3 ±1.8	0.909
P_aO_2, kPa	13.3 ±0.846	13 ±1.04	0.340
P_aCO_2, kPa	5.06 ±0.686	4.54 ±0.888	0.069
V_D/Vt, %	13.1 ±4.2	15.6 ±4.2	0.092
A-aDO_2, kPa	2.75 ±0.62	3.30 ±0.92	0.046
Δ A-aDO_2, kPa	0.95 ±0.44	1.61 ±0.92	0.009

Definition of abbreviations: $\dot{V}O_2$= oxygen consumption; $\dot{V}_E$= minute ventilation; MVV= maximum voluntary ventilation; $\dot{V}_E/\dot{V}CO_2$= ventilatory equivalent for carbon dioxide; S_pO_2= oxygen saturation measured by pulse oximetry; RR= respiratory rate; V_T= tidal volume; IC= inspiratory capacity; IRV=inspiratory reserve volume; RER= respiratory exchange ratio; P_aO_2= arterial oxygen tension; P_aCO_2= arterial carbon dioxide tension; V_D/V_T= dead-space ratio; A-aDO_2= alveolar-arterial oxygen gradient; Δ A-aDO_2= change in alveolar-arterial oxygen gradient from rest to peak exercise.

All values are expressed as mean±SD unless otherwise stated.

Table 3: Multivariable linear regression model for $\dot{V}O_2$ (ml/min/kg) prediction

	B- coefficient	**95% CI**	**P value**
Age	*-0.483*	*-0.749 – -0.217*	*0.001*
Gender (male)	*1.146*	*-2.478 – 4.770*	*0.523*
DL_{CO}% pred.	*0.284*	*0.143 – 0.426*	*<0.0005*
T_{70}	*0.059*	*0.027 – 0.091*	*0.001*
Constant	*21.012*	*5.141 – 36.883*	*0.011*

Definition of abbreviations: $\dot{V}O_2$= oxygen consumption; DL_{CO}% pred.= lung diffusing capacity for carbon monoxide as a percentage of predicted; T_{70}= Time to 70% of the initial force-time integral.

Adjusted R^2 = 0.602, p<0.0005