BRIEF COMMUNICATION / COMMUNICATION BRÈVE

Skeletal muscle capillarization and oxidative metabolism in healthy smokers

Rob C.I. Wüst, Richard T. Jaspers, Willem J. van der Laarse, and Hans Degens

Abstract: We investigated whether the lower fatigue resistance in smokers than in nonsmokers is caused by a compromised muscle oxidative metabolism. Using calibrated histochemistry, we found no differences in succinate dehydrogenase (SDH) activity, myoglobin concentration, or capillarization in sections of the vastus lateralis muscle between smokers and nonsmokers. The relationship between fatigue resistance and SDH activity in nonsmokers ($r = 0.93$; $p = 0.02$) is absent in smokers. This indicates that the lower muscle fatigue resistance of smokers can likely be attributed to causes other than differences in oxidative metabolism and capillarization.

Key words: skeletal muscle, smoking, myoglobin, oxidative capacity, oxygenation, capillarization.

Résumé : Nous testons l'hypothèse selon laquelle la résistance moins grande des fumeurs à la fatigue est due à un compromis au niveau des substrats oxydés dans le métabolisme des muscles. Au moyen d'une technique histochimique étalonnée, nous n'observons aucune différence d'activité de la succinate déshydrogénase (SDH), de la concentration de myoglobine et de la capillarisation dans le muscle vaste externe des fumeurs et des non-fumeurs. La relation chez les non-fumeurs entre la résistance à la fatigue et l'activité de la SDH ($r = 0{,}93$; $p = 0{,}02$) n'est pas observée chez les fumeurs. La résistance moins grande à la fatigue des fumeurs est vraisemblablement due à d'autres causes que des différences de métabolisme oxydatif et de capillarisation.

Mots-clés : muscle squelettique, usage du tabac, myoglobine, capacité oxydative, oxygénation, capillarisation.

[Traduit par la Rédaction]

Introduction

Chronic obstructive pulmonary disease (COPD) is associated with reduced exercise tolerance and, in general, reduced muscle fatigue resistance (Gosselink and Decramer 1998; Wüst and Degens 2007). The main determinants of skeletal muscle fatigue resistance are: oxygen delivery to the muscle, oxygen transport from interstitial space to the core of the muscle fibers, fiber-type composition, and oxidative capacity (Degens and Veerkamp 1994), which is proportional to succinate dehydrogenase (SDH) activity (Van der Laarse et al. 1989). The oxygenation of muscle tissue is largely dependent on capillary density (Hoofd et al. 1985), myoglobin concentration (Richardson et al. 2001), and fiber cross-sectional area (Hill 1965). In patients with COPD, muscle fiber atrophy and a reduction in capillarization and oxidative capacity all negatively affect muscle fatigue resistance (Maltais et al. 1996; Jobin et al. 1998; Wüst and Degens 2007; Green et al. 2008). As cigarette smoking is associated with over 80% of the COPD cases (Yawn and Kaplan 2008), smoking may be an important factor in the muscle adaptations that compromise the strength and fatigue resistance of COPD patients; skeletal muscle fatigue resistance is already lower in young, healthy smokers than in age- and physical-activity-matched nonsmokers (Morse et al. 2007). Although inhaled carbon monoxide (CO), a constituent of cigarette smoke, that reached 6% carboxyhemoglobin (COHb) resulted in an 8% reduction in the fatigue resistance of nonsmoking subjects (Morse et al. 2008), it is not clear whether this is the sole explanation. Given the determinants of skeletal muscle fatigue resistance described above, we hypothesized that in skeletal muscle mitochondrial density, capillary density and (or) myoglobin concentrations would

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be lower in smokers, and (or) that the proportion of fast muscle fibers would be higher in the skeletal muscle of smokers than of nonsmokers.

Materials and methods

Participants

Five nonsmokers (2 men and 3 women) and 5 smokers (2 men and 3 women) participated in this study. Exclusion criteria were known cardiovascular, neuromuscular, or respiratory diseases. Written informed consent was obtained from each participant prior to testing. All procedures were approved by the local ethics committee of Manchester Metropolitan University (Manchester, U.K.). The age of the nonsmokers (range, 23–72 years; median, 45 years) and smokers (range, 25–72 years; median, 40 years) was similar. The smokers smoked, on average, 15 ± 9 cigarettes·day^{-1}, while the nonsmokers never smoked. Mean cigarette pack-years (packs smoked·day^{-1} × the number of years of smoking) was 12.9 (median, 9.9; range, 2.6–35.0 pack-years). The physical activity score of the subjects, assessed by questionnaire (Baecke et al. 1982), was 8.1 ± 1.3 and 7.1 ± 1.2 for the nonsmokers and smokers, respectively (p = 0.20). A physical activity score of <6 indicates a sedentary lifestyle, whereas scores >9 represent a high level of activity.

Fatigue resistance and anatomical cross-sectional area

Fatigue resistance of the quadriceps muscle was determined using electrical stimulation (2 min; 1 s at 30 Hz on, 1 s off; constant stimulus intensity corresponding to 22 ± 4% of maximum voluntary force in the nonfatigue state). The procedures are detailed in Morse et al. (2007). Anatomical cross-sectional area (ACSA) was measured with magnetic resonance imaging (Morse et al. 2007).

Skeletal muscle biopsy

A percutaneous biopsy of the vastus lateralis muscle was obtained from each participant, using a conchotome. The site of the biopsy was anesthetized with 2% lidocaine, and a 1 cm incision was made at ~20 cm above the patella. The biopsy was frozen in liquid nitrogen and stored at –80 °C.

Enzyme histochemistry

Transverse 10 μm sections were cut in a cryostat at –20 °C. Sections were stained for myofibrillar adenosine triphosphatase (mATPase), after preincubation at pH 4.7, to classify fibers as type I or type II (Brooke and Kaiser 1970). Serial sections were incubated for myoglobin concentration (Van Beek-Harmsen et al. 2004) and SDH activity (Bekedam et al. 2003), which is proportional to the maximum rate of oxygen consumption when oxygen is not rate-limiting (Van der Laarse et al. 1989). SDH activity is given as the absorbance increase at 660 nm per micrometer section thickness and per second of incubation time (ΔA_{660}·μm^{-1}·s^{-1}). Myoglobin concentration was assessed by converting the absorbance at 436 nm to concentration, using a calibration line (Van Beek-Harmsen et al. 2004).

Capillarization

Another serial section was stained with lectin to depict capillaries (Ahmed et al. 1997). Capillarization was analyzed with the method of capillary domains described by Degens et al. (1992). This method gives not only the overall indices of capillary density and capillary to fiber ratio, it also takes into account the presence of different fiber types and the heterogeneity in capillary spacing (standard deviation of the log-transformed domain radius (Log_RSD)). Domains were defined as the area surrounded by a capillary delineated by equidistant boundaries from adjacent capillaries. The fiber cross-sectional area (FCSA) was determined by tracing the outlines of the fibers on a digitizing tablet (Summagraphics Digitizers, Austin, Tex.). The capillary supply to a fiber was given as the local capillary to fiber ratio (LCFR), calculated as the sum of the fractions of the capillary domains overlapping a given fiber. The capillary fiber density (in mm^{-2}) was calculated as the LCFR/FCSA.

Statistical analysis

We used multilevel analyses to evaluate differences between smokers and nonsmokers (MLwiN, version 2.0, Bristol, U.K.). This method incorporates nominal variables (Twisk 2006), as well as variance at the level of the cell and the individual. Slow and fast fibers were analyzed separately, and gender, age, and physical activity were included as covariates. Significance level was set at $p < 0.05$. Unless otherwise stated, values are given as means ± standard error of the mean (SEM).

Results

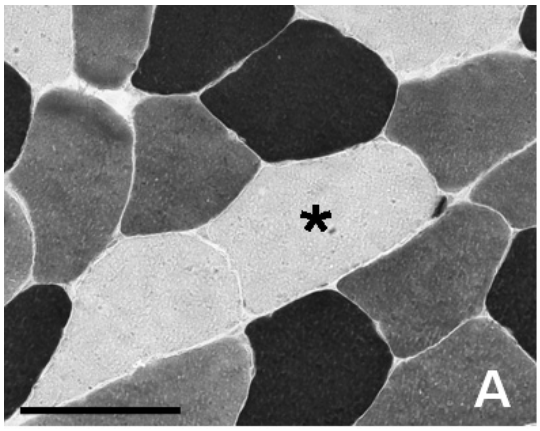
The correlation coefficient between the physical activity score and the fiber-type weighted mean SDH activity was 0.87 for nonsmokers (p = 0.06) and 0.88 for smokers (p = 0.01). All participants had a normal respiratory function (data not shown).

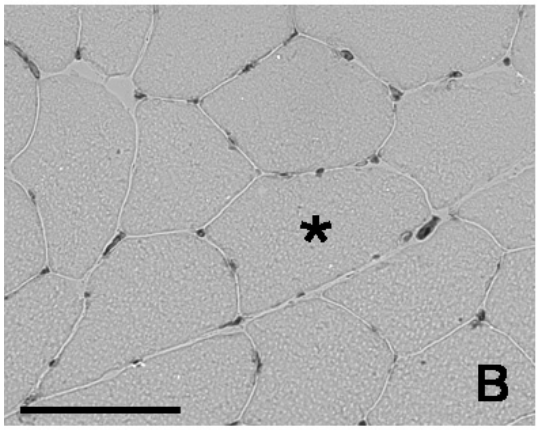
Maximal strength and fatigue resistance

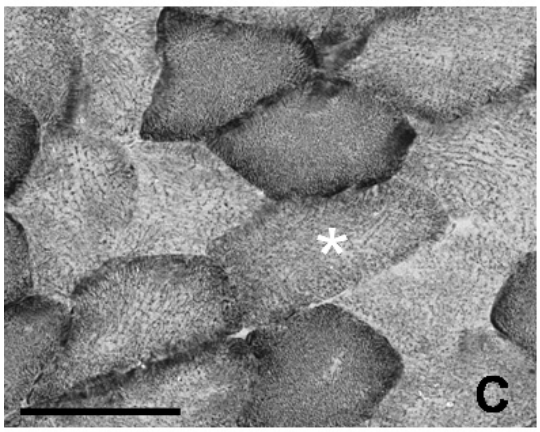
Maximal torque and ACSA were not different in nonsmokers and smokers, with 201 ± 24 vs. 203 ± 35 Nm (p = 0.96) and 61 ± 4 vs. 54 ± 6 cm^2 (p = 0.32) for torque and ACSA, respectively. Fatigue resistance, however, was significantly reduced by smoking; torque dropped to 77.4 ± 4.5% vs. 61.4 ± 4.5% of initial value in nonsmokers and smokers, respectively ($p < 0.05$). The correlation coefficient between physical activity levels and fatigue index was 0.83 (p = 0.08) and –0.88 (p = 0.05) for the nonsmokers and smokers, respectively. No significant relationship was observed between cigarette smoking history and the fatigue index; correlation coefficients were 0.73, –0.32, and 0.45 for cigarettes·day^{-1}, years of smoking, and cigarette pack-years, respectively.

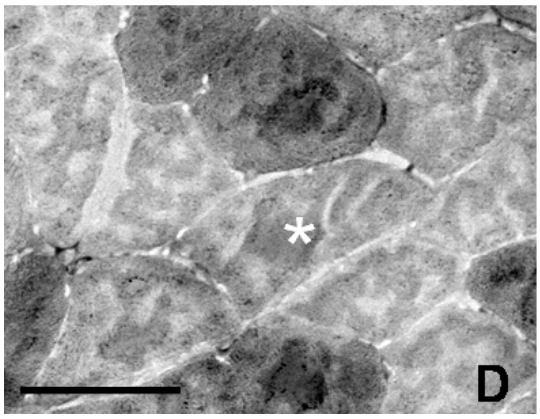
Muscle biopsies

On average, 160 (SD, 45) fibers were analyzed for each subject. Figure 1 shows examples of mATPase staining (Fig. 1A), capillaries (Fig. 1B), SDH activity (Fig. 1C), and myoglobin concentration (Fig. 1D). The fiber-type composition was similar in smokers and nonsmokers, with 55 ± 4% vs. 59 ± 11% and 45 ± 4% vs. 41 ± 11% for type I and II, respectively. Although no differences were observed in ACSA, mean FCSA was 25% lower in smokers than in nonsmokers (p = 0.001; Fig. 1E). Capillarization, in terms of









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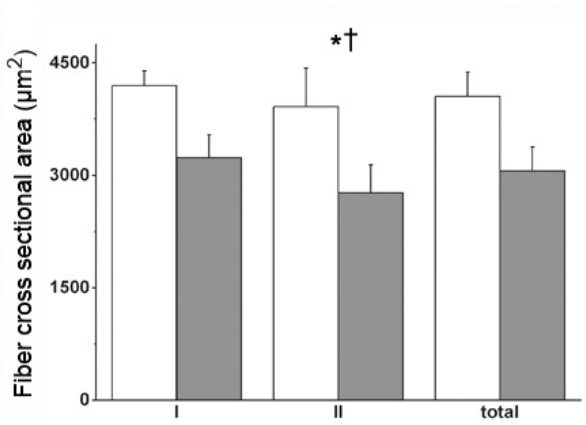

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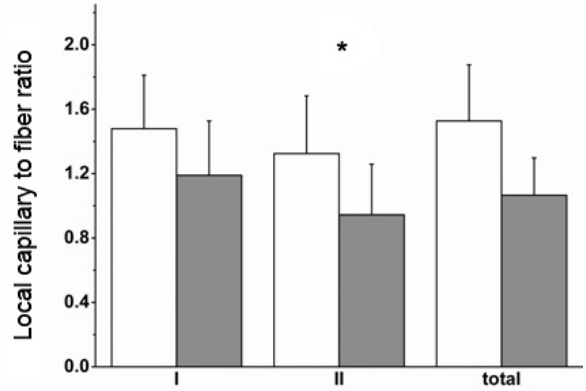

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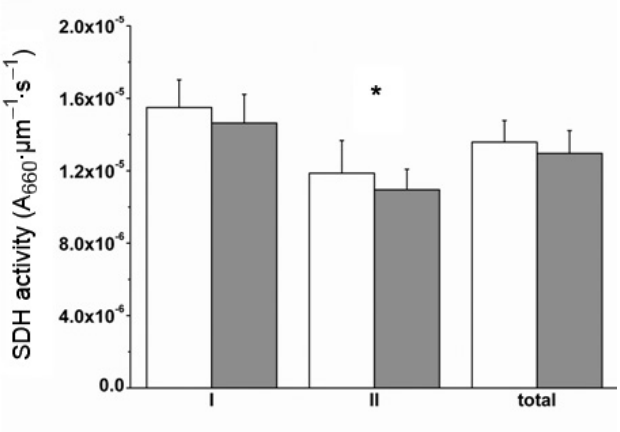

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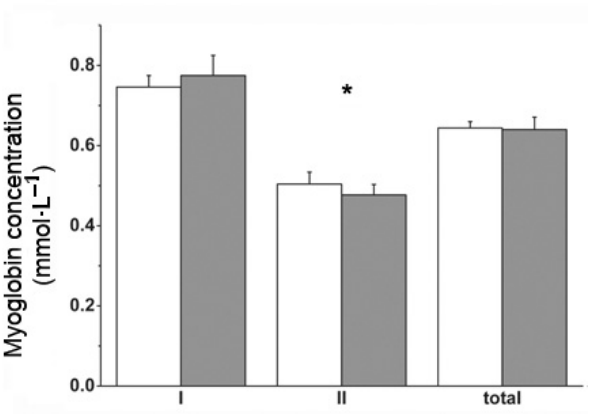


Fig. 1. Serial cross-sections of a representative sample and results of fiber types and fiber cross-sectional area (A and E); capillary staining and local capillary to fiber ratio, or capillary to fiber ratio for total (B and F); calibrated succinate dehydrogenase (SDH) activity (C and G); and myoglobin concentration (D and H). The white bars represent the nonsmokers and the grey bars represent the smokers. Total is the weighted average of the type I and II cells. Bars in photomicrographs indicate 100 μm and asterisks (*) denote similar fiber. Values are means ± SEM. In (E) to (H), asterisks (*) and dagger (†) denote statistical differences between type I and II and between smokers and nonsmokers, respectively.

LCFR, for both fiber types and overall capillary to fiber ratio did not differ between smokers and nonsmokers, with 1.07 ± 0.23 vs. 1.53 ± 0.35 for smokers and nonsmokers, respectively (Fig. 1F), but was higher in type I than type II fibers ($p < 0.001$). Capillary fiber density, however, was similar for type I and type II fibers, and did not differ between smokers and nonsmokers (data not shown). Similarly, the overall capillary density did not differ significantly between smokers and nonsmokers (314 ± 72 vs. 333 ± 60 mm^{-2}). The $log_R SD$ was also similar in both groups, with 0.108 ± 0.016 vs. 0.098 ± 0.009 for smokers and nonsmokers, respectively. SDH activity (Fig. 1G) was higher in type I than type II fibers ($p < 0.001$). However, the SDH activity and FCSA × SDH activity (integrated SDH activity) of each fiber type were similar in the 2 groups, indicating a similar total oxidative capacity of the muscle cells in smokers and nonsmokers. The myoglobin concentration was higher in type I than type II cells ($p < 0.001$), but was not significantly altered by smoking (Fig. 1H).

Interestingly, the correlation coefficients of the fatigue resistance and overall SDH activity was 0.93 ($p = 0.02$) in controls, but was not significant in smokers ($r = –0.67$; $p = 0.21$) (Fig. 2). The slope of the relationship between SDH activity and fatigue resistance was significantly different between smokers and nonsmokers, with 3.56 (0.8) × 10^6 vs. –2.4 (1.5) × 10^6% × s × μm × A_{660}^{-1} for nonsmokers and smokers, respectively ($p = 0.01$).

The correlation of the percentage of type I fibers and the fatigue resistance in the nonsmokers was 0.97 ($p = 0.01$), while it was not significant in the smokers ($r = –0.60$, $p = 0.28$).

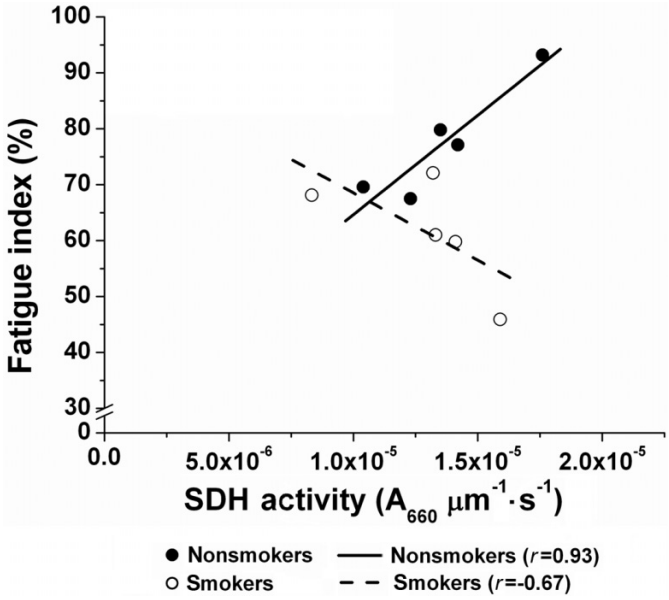
No significant relationships were observed between capillarization or myoglobin concentration and fatigue resistance in either group. Neither were any parameters (percentage type I, SDH activity, capillarization, myoglobin) related to smoking history, in terms of cigarettes per day or cigarette pack-years (data not shown).

Discussion

The main observation of this study is that the lower fatigue resistance in smokers, compared with nonsmokers (Morse et al. 2007), is not related to differences in fiber-type composition, oxidative capacity, myoglobin concentration, or capillarization. The correlation between SDH activity and fatigue resistance in nonsmokers was lost in smokers. These data suggest that the reduced fatigue resistance in smokers is not due to alterations in determinants of oxidative metabolism, but may be related to factors in cigarette smoke that affect the delivery of oxygen and the function of the respiratory chain.

We took special care to match the physical activity level of the nonsmokers and smokers. This is important, as there is a positive correlation between physical activity and SDH activity (Den Hoed et al. 2008), which we also observed in our data ($r = 0.83$). The absence of a significant relationship between myoglobin concentration or capillary density and fatigue resistance in the vastus lateralis muscle of smokers and nonsmokers suggests that these parameters are not determinants of the fatigue resistance elicited by our protocol, using electrical stimulation. Interestingly, the SDH activity did not correlate with fatigue resistance in smokers (Fig. 2).

Fig. 2. Effects of smoking on the relation between succinate dehydrogenase (SDH) activity and fatigue index. The fatigue index is the torque at the end of the fatigue resistance test, expressed as a percentage of the initial torque. A linear relationship between SDH activity and fatigue resistance was found for the nonsmokers ($p = 0.02$), but was absent in the smokers ($p = 0.21$).



Because of the small sample size, there is a risk for false negative effects. We minimized this risk by selecting subjects for age, gender, and physical activity level, and by using multilevel analysis. In addition, power analysis revealed that for a power of 0.8 for most of the parameters (except for LCFR), more than 50 subjects were required to reach significance for nonsignificant differences in Fig. 1, indicating that the differences between the means of these parameters are small anyway, and unlikely to explain the significant difference in fatigue resistance.

Cigarette smoking has been associated with an enhanced expression of factors involved in muscle protein degradation (Petersen et al. 2007) and type I fiber atrophy (Montes de Oca et al. 2008). Although the decline in ACSA was not significant, we observed that the FCSA of both type I and type II fibers in smokers was significantly smaller than in nonsmokers. This reduction in FCSA would result in reduced oxygen diffusion distances from the periphery to the

centre of the fiber (Hill 1965), which may delay the development of fatigue. Reduced FCSA is associated with a lower minimal interstitial oxygen tension, which prevents an anoxic core in maximally working fibers ($PO_{2\,crit}$), calculated with Hill's oxygen diffusion model, including myoglobin-facilitated oxygen diffusion (for details, see Van Beek-Harmsen et al. (2004)), in the type I and type II fibers in smokers than non-smokers, with 9.1 ± 1.8 vs. 6.8 ± 3.4 mm Hg in type I and 6.6 ± 1.7 vs. 4.3 ± 2.6 mm Hg in type II for nonsmokers and smokers, respectively. Despite this (nonsignificant) 25% to 34% reduction in $PO_{2\,crit}$ at the level of the muscle fiber, overall fatigue resistance was lower in the smokers.

Taken together, the results of this study suggest that other factors determining oxygen supply and (or) the ability to utilize oxygen are affected by smoking and limit muscle performance in smokers. A diminished oxygen supply may occur when COHb is formed; the inhalation of CO until COHb reached 6% caused an 8% reduction in fatigue resistance (Morse et al. 2008). Because the $PO_{2\,crit}$ is reduced and the mean blood COHb level in smokers is reported to vary from 4%–5% (Hampson et al. 2006), less than half of the difference in the fatigue index between smokers and nonsmokers can be explained by this effect of CO. However, CO also causes a left-shift of the HbO_2 dissociation curve, hampering the release of oxygen, and it impairs the facilitated transport of oxygen within the muscle cell by binding to myoglobin (Gorman et al. 2003). Finally, CO inhibits the function of complex IV of the electron transport chain (Alonso et al. 2003). A combination of these factors attenuates not only delivery but also utilization of oxygen. The delivery of oxygen may further be hampered by an impaired peripheral blood flow in smokers (Ronnemaa et al. 1999), due to endothelial dysfunction through oxidative stress and the reduced bioavailability of nitric oxide (Ambrose and Barua 2004; Montes de Oca et al. 2008). The nature and relative contribution of each of these factors in determining smoking-related fatigue, however, warrant further investigation.

We conclude that while muscle fiber size was smaller in the smokers, fiber-type distribution, capillarization, and SDH activity of the muscle are similar in physical-activity-matched smokers and nonsmokers. We suggest that the main cause of increased peripheral fatigue may be impaired oxygen delivery and (or) utilization by the muscle through acute effects of smoking, such as those conveyed by CO in cigarette smoke.

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