Quadriceps muscle strength in scoliosis

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Key words:

extra-pulmonary restrictive lung disease; chronic obstructive pulmonary disease; oxidative stress; quadriceps muscle weakness

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ABSTRACT

Quadriceps muscle weakness is an important component of COPD. We hypothesised that quadriceps weakness would also be a feature of restrictive lung disease due to scoliosis.

We studied 10 patients with severe scoliosis (median (interquartile range, IQR) FEV₁ 35.3 (11) % predicted), 10 patients with severe, COPD (median (IQR) FEV₁ 26.5 (9.0) % predicted) and 10 healthy age matched adults. We measured quadriceps strength, exercise capacity and analysed quadriceps muscle biopsies for myosin heavy chain (MyHC) isoform expression and the presence of oxidative stress.

Both groups exhibited quadriceps weakness with median (IQR) maximal voluntary contraction force being 46.0 (17.0) kg, 21.5 (21.0) kg and 31.5 (11.0) kg, respectively (p=0.02 and 0.04 respectively for each patient group against controls). Oxidative stress was significantly greater in the quadriceps of both restrictive and COPD patients. The scoliosis patients exhibited a decrease in the proportion of MyHC Type I compared with controls; median (IQR) 35.3 (18.5)% compared with 47.7 (9.3)%, p=0.028. The scoliosis patients also showed an increase in MyHC IIX (median (IQR) 26.3 (15.5)% compared with 11.3 (13.0)%, p=0.01.

Quadriceps weakness is a feature of severe scoliosis; the similarities between patients with scoliosis and patients with COPD suggest a common aetiology to quadriceps weakness in both conditions.
INTRODUCTION

In patients with advanced Chronic Obstructive Pulmonary Disease (COPD) quadriceps wasting and weakness are both features which suggest a poor prognosis (1, 2), but few data exist concerning quadriceps weakness in other chronic pulmonary diseases and, to our knowledge, none on the underlying muscle phenotype and molecular changes. Although pulmonary rehabilitation (PR) is of benefit in both patients with restrictive lung disease (3) and COPD (4), most investigators agree that precise understanding of the causes of quadriceps weakness in pulmonary disease might allow the development of therapies that could be offered to patients as an adjunct to PR or to patients who are unable to participate in PR.

In addition to weakness several biopsy-based observations are recognised as components of the COPD quadriceps phenotype. These include oxidative stress (5) along with a fibre type switch to a less resistant phenotype (6). Since scoliosis, like COPD, when severe causes exercise limitation and respiratory failure we considered that we might also observe quadriceps weakness in patients with scoliosis and that they might exhibit a peripheral muscle phenotype which resembled that of patients with COPD.

METHODS

Subjects
10 patients with idiopathic scoliosis and 10 patients with severe COPD were recruited from the home ventilation and advanced emphysema clinics at the Royal Brompton Hospital. The scoliotic patients had been referred for assessment of symptoms that might be attributable to nocturnal hypoventilation in the presence of reduced spirometry. COPD patients had been referred for this purpose or for consideration of lung volume reduction surgery or lung transplantation. Subjects were stable at the time of the study and were on standard treatment for their lung disease including, where appropriate, bronchodilators and inhaled corticosteroids, but not non-invasive ventilation which was the reason for referral. No patients were on long-term oral steroids. Two of the COPD patients had received short courses of oral prednisolone as treatment for exacerbation; most recently 4 and 5 months respectively prior to being studied. Exclusion criteria included cardiovascular disease, chronic metabolic diseases, suspected para-neoplastic or myopathic syndromes, and/or treatment with drugs known to alter muscle structure and/or function. Five patients in the COPD group were on long-term domiciliary oxygen as opposed to 1 in the scoliosis group. Ten healthy age matched control subjects recruited from a register in our laboratory were also studied. The Royal Brompton Hospital Research Ethics Committee approved the study and all subjects provided written, informed consent.

**Nutritional and physiological measurements**

Anthropometric measurements, fat free mass determined by bioelectrical impedance analysis (7) and pulmonary function tests were recorded in both
patients and controls. Patients in the restrictive group had their arm span distance used instead of height since it was greater (8). The patient groups also completed a six minute walk test (6MW) repeated on 2 occasions; the highest result obtained was used for analysis.

**Quadriceps force measurements**

Subjects were studied supine, with the knee flexed at 90° over the end of the bed, in a modification of the method of Edwards and co-workers (9); the dominant leg was studied and signals recorded using apparatus previously described by us (10). Quadriceps strength was measured by supramaximal magnetic stimulation of the femoral nerve (TwQ) and by maximal isometric voluntary contraction (QMVC) following a 20 minute rest period for depotentiation (11). Femoral nerve stimulation (12) was performed with a double Magstim 200 magnetic stimulator (Magstim Co, Dyfed, Wales) discharging both units simultaneously through a 70 mm ‘branding iron’ coil. The optimal coil position was determined at the start of the experiment; supramaximality was confirmed in each subject by recording the unpotentiated Tw Q at a range of stimulus intensities between 70 and 100% output. A minimum of 7 stimuli at 100% were then performed and TwQ defined as the mean of these recordings. QMVC was performed so that the force generated was visible to subject and investigator for positive feedback. Efforts were sustained for at least 5 seconds. Subjects rested for 30 seconds between each contraction to minimize ‘twitch-on twitch’ potentiation (13). A minimum of 3 efforts were made with vigorous encouragement until there was
no improvement in performance; the biggest effort recorded was used for analysis.

**Quadriceps muscle biopsy**

A needle biopsy of the vastus lateralis of the contralateral (i.e. non-dominant) leg was performed in all subjects (14). The samples were immediately frozen in liquid nitrogen and stored at -80°C until further processing.

**Muscle biopsy analyses**

Full details may be found in the online depositary but, in brief, we performed analysis for myosin heavy chain isoform expression in Maastricht using previously described methods (15). We also sought evidence of oxidative stress in muscle proteins and lipids in Barcelona using previously described methods (5, 16, 17)

**Statistical analysis**

Data are expressed as the median ± interquartile range unless otherwise stated. Differences between the 3 groups were explored using the Kruskal-Wallis test followed by the Mann-Witney test since it could not be safely assumed that the data were normally distributed. A p-value of <0.05 was taken to be significant. A statistical software package was used for all calculations (Statview; SAS Institute; Cary, NC).
RESULTS

Characteristics of the study subjects

The characteristics of the subjects are shown in Table 1. The patient and control groups were well matched with respect to age. Fat free mass index did not differ between groups but the BMI was reduced in scoliotic patients compared to both controls and COPD patients \((p=0.03\) for both). There was significant but comparable lung function abnormality, judged by \(FEV_1\), in both patient groups with a median (IQR) \(FEV_1\) 35.3 (11) % predicted) for the scoliotic patients and a severe obstructive defect in the COPD group (median (IQR) \(FEV_1\) 26.5 (9.0) % predicted). As expected (Table 1) the restrictive patients had a high ratio of \(FEV_1/VC\) (0.80) and the COPD patients had a low ratio (0.31). All participants were non-smokers, with the exception of 1 scoliotic and 1 COPD patient; all the COPD patients had been smokers as had 3 other scoliotic patients and 7 of the controls.

Quadriceps force measurement

Quadriceps strength differed significantly between groups judged by the Kruskal-Wallis test when assessed using either twitch force \((p=0.03)\) or maximal voluntary contraction \((p=0.05)\) (Figure 1). Direct group comparisons showed that the scoliotic patients were not statistically different from the COPD group (Table 1), but quadriceps strength was also reduced in both
scoliosis and COPD patients compared with controls judged by MVC (p=0.02 and p=0.04 respectively) and, in the case of restrictive patients, by TwQ (p=0.013).

**Muscle biological analyses**

Results corresponding to myosin heavy chain isoform expression, oxidative and redox balance are shown in Table 2. Analysis of myosin heavy chains showed significant differences in the proportions of Type IIa (P=0.011), Type IIx (p=0.03) and Type I (p=0.001) myosin, explained by a significant difference in the proportions of type I and IIa myosins between healthy subjects and COPD patients (p=0.009 and p=0.0009 respectively) and by significant differences between the scoliosis patients and controls (Type I Myosin p=0.028, Type IIx p=0.01). Overall the scoliosis group, had a similar MyHC Type I profile as the COPD group (Figure 2).

The oxidative stress markers MDA-protein adducts (Figures 3 and 4), protein tyrosine nitration (Figures 3 and 5) and protein carbonylation differed significantly between groups (p=0.001, p=0.001 and p=0.05 respectively). These differences were explained by a significant increase in the quadriceps of scoliosis patients in MDA-protein adducts (p=0.002), protein tyrosine nitration (p=0.001) and protein carbonylation (p=0.04). Statistically significant increases in 2 of these variables were also observed in COPD patients (MDA-protein adducts and protein tyrosine nitration, p=0.002 and p=0.008, respectively). The antioxidant mechanism Mn-SOD but not catalase differed
between the 3 groups (Table 2) and was increased in scoliotics compared with COPD patients (p=0.01).

Indices of oxidative stress were compared with physiological data. No significant responses were observed except a modest negative relationship observed between quadriceps strength measured as maximal voluntary contraction force and protein tyrosine nitration ($\rho=-0.46, p=0.027$).

DISCUSSION

The main finding of the present study is that patients with very severe scoliosis display marked quadriceps weakness with a reduction in exercise performance judged by the 6MW to approximately $\frac{2}{3}$ of expected values. In addition to a comparable degree of weakness we observed, based on muscle biopsy data strong similarities between scoliotics and COPD patients; both groups had a reduction in Type I myosin and evidence of oxidative stress suggesting a common aetiological mechanism.

Critique of the Method

To some extent the present study is exploratory in nature and therefore has some limitations. Some investigators have hypothesised that COPD is a specific inflammatory disorder triggered and modulated by tobacco smoking and that the quadriceps weakness follows downstream from this inflammation (e.g. Ref (18)). If that were the case then quadriceps weakness would not, of
necessity, be present in non-inflammatory respiratory conditions such as scoliosis. Whilst our data, in this context, argue against an inflammatory aetiology to peripheral muscle weakness in COPD we acknowledge that blood markers of inflammation, such as IL-6, IL-8 or TNFα were not measured in the present study. Interestingly our consortium (19) and others (20) have recently demonstrated that these inflammatory markers are in any case under rather than over represented in the limb muscle of patients with COPD.

Considering patients with COPD and healthy adults, our study appears small. However patients with severe scoliosis who are naïve to non-invasive ventilation are relatively uncommon; in fact we were unable to find a prior study in the literature from patients with incipient respiratory failure with this condition. We might have enlarged our cohort by including, for example, patients with pulmonary fibrosis but we elected to confine the restrictive group to those with an extra-pulmonary and non-neurological cause for respiratory failure in order to get data that was as ‘clean’ as possible.

A further limitation of our study is that measures of muscle bulk, for example by mid thigh CT cross-sectional area (1) were not available. Thus it cannot be assumed that weakness is synonymous with atrophy although most data, including a recent study from our group using ultrasound (21), suggest that muscle bulk is the primary determinant of strength (22). Unfortunately we did not have 6MW data on our healthy control subjects however the expected 6MW distance for in our control group would be 574m (23); therefore both
patient groups may be considered to have, as expected, a substantial and clinically relevant reduction in exercise capacity.

**Significance of the findings**

Our data demonstrate severe skeletal muscle weakness in patients with extrapulmonary restrictive lung disease, accompanied by a change in myosin heavy chain isoform expression. Although not directly measured we assume weakness to be associated with muscle wasting; i.e. loss of muscle bulk (21). Few data on this topic exist although quadriceps weakness was also observed in the few restrictive patients who participated in a previous study investigating the effect of non-invasive mechanical ventilation (24). In addition, if they complete the course, pulmonary rehabilitation is effective for such patients (3), consistent with our finding that quadriceps weakness is present.

The Barcelona group and other investigators (5, 25, 26) have already argued that oxidative and nitrosative stress are increased in the muscles of patients with COPD. Indeed, oxidative stress has been proposed as one of the most important mechanisms involved in the aetiology of peripheral muscle dysfunction in COPD. In the current study we again observed that lower limb muscles of patients with COPD undergo severe protein carbonylation and nitration. However we extend them importantly by showing similar findings in the *vastus lateralis* of patients with scoliosis. Since these patients also had quadriceps weakness our data suggest the hypothesis that both oxidative and
nitrosative stress were also involved in the peripheral muscle dysfunction of the scoliosis patients; although it is acknowledged that our data do not demonstrate a causal role. Interestingly, when considering the entire dataset a weak inverse relationship was observed between increasing protein tyrosine nitration and increasing muscle weakness (data not shown). Montes de Oca and colleagues (27) have recently reported evidence of increased nitric oxide end-products in smokers, but this cannot explain our observations in patients with restrictive disease since they were, in 9 of 10 instances, non-smokers.

We believe the present study to be the first in which quadriceps biopsy has been undertaken in patients with Type II respiratory failure due to scoliosis; interestingly however quadriceps biopsy of patients with scoliosis who were not in respiratory failure was undertaken by Maffuli (28) who found ‘a mild dominance’ of type I fibres in contrast with the current results. His patients were referred for spinal surgery rather than because of suspected Type II respiratory failure and therefore disease severity or age may explain this discrepancy. A recent meta-analysis (29) has confirmed frequent prior observations (30-32) that Type II fibres predominate in patients with advanced COPD, an observation repeated in the present study. Our data show that, in patients with scoliosis, with a degree of weakness and respiratory impairment which equalled or exceeded that seen in patients with COPD myosin shifts in myosin heavy chain isoform expression also occur. Overall the pattern in scoliosis patients resembled COPD patients, in that Type I myosin heavy chain isoform expression was reduced, but we note that the magnitude of the reduction was less than in COPD patients and that in scoliotics the
‘beneficiary’ was Type IIx myosin heavy chain isoform expression whereas in COPD MyHC IIa was increased. The reason for this discrepancy is unclear and not provided by the present data but since MyHCIIx is viewed by some investigators as an adaptive response one could speculate that this reflects the more profound strength loss and the longer history of preceding disease typically seen in scoliosis.

These data suggest that factors common to scoliosis and COPD are driving fibre type shift. The pathophysiological mechanism remains to be elucidated and candidate mechanisms will need to be evaluated in future studies. One striking finding in the present study was the reduced body mass index in the scoliotic patients; it has recently been noted elsewhere that BMI is disproportionately reduced in paediatric scoliotic patients with the most severe pulmonary restriction (33), although we acknowledge that these patients were very much younger than our own. However similar changes were observed in COPD patients who did not have a reduced BMI and it has also been established that the prevalence of quadriceps weakness in COPD exceeds that of fat free mass depletion (34, 35). Comparison with COPD, for which more data is available, suggests that reduced physical activity could also be a candidate mechanism although this was not measured in the present study. Fibre type changes are clinically relevant because they predispose, in a manner related to its severity, to leg discomfort and low frequency fatigue during exercise (36). Our data suggest that this mechanism could also be operative in scoliotics.
Factors which our COPD patients might have been exposed to, even though studied in a stable condition include systemic inflammation, drugs (including corticosteroids) used to treat prior exacerbations and associated co-morbidities such as peripheral vascular disease. These factors were not systematically evaluated in the present cohort, though no participants were using oral steroids when biopsied, or indeed for a minimum of 4 months beforehand. Corticosteroids are known to be associated with muscle atrophy through a myostatin dependent mechanism (37) which increases expression of atrogenes including atrogin and MURF-1. However, steroid induced muscle atrophy classically preferentially involves type II fibres so this, coupled with the lack of recent exposure in our cohort, seems an unlikely mechanism. It has already been noted that systemic inflammation was not evaluated in the present study. Patients with COPD, to a greater extent than patients with scoliosis disease experience exacerbations; quadriceps strength is decreased during exacerbation (38). Data concerning the effect of exacerbation frequency on quadriceps strength and its relationship with loss of fat free mass is presently sparse (35), but only two of our COPD patients had had exacerbations in the previous year, and none in the preceding 3 months. Lastly hypoxemia, at least at rest, seems an unlikely explanation of our findings since the median PaO₂ in scoliotics was 9.1 kPa. However we acknowledge that, like COPD patients, scoliotic patients develop exercise induced hypoxia (39); this was not measured in the present study and cannot be excluded as a potential trigger.
In conclusion, we provide the first data documenting the function and content of the quadriceps muscle in patients with advanced scoliosis and compare them to both COPD patients and healthy adults. Important similarities were weakness of the quadriceps muscle, reduced type I myosin and the presence of oxidative stress, suggesting a common aetiological mechanism in both disease groups.
Acknowledgements

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Table 1: Subject demographics

<table>
<thead>
<tr>
<th></th>
<th>Control Group (10)</th>
<th>Scoliosis Group (10) Median (IQR)3M/7F</th>
<th>COPD Group (10) Median (IQR) 10 M</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age</strong></td>
<td>61 (13)</td>
<td>63.5 (6.0)</td>
<td>65.0 (13.0)</td>
</tr>
<tr>
<td><strong>Height/arm span (m)</strong></td>
<td>1.76 (0.9)</td>
<td>1.62 (0.34)¥*</td>
<td>1.75 (0.6)</td>
</tr>
<tr>
<td><strong>Weight (kg)</strong></td>
<td>81.1 (13.8)</td>
<td>57.9 (27.7)¥*</td>
<td>86.3 (24.4)</td>
</tr>
<tr>
<td><strong>BMI (kg/m²)</strong></td>
<td>26.0 (4.0)</td>
<td>22.4 (9.1)¥*</td>
<td>27.6 (5.9)</td>
</tr>
<tr>
<td><strong>FFMI (kg/m²)</strong></td>
<td>18.0(2.3)</td>
<td>14.9 (7.5)</td>
<td>17.7 (1.9)</td>
</tr>
<tr>
<td><strong>FEV₁ % predicted</strong></td>
<td>89.5 (33.0)</td>
<td>35.3 (11.0)*</td>
<td>26.5 (9.0)*</td>
</tr>
<tr>
<td><strong>FVC % predicted</strong></td>
<td>90.0 (20.0)</td>
<td>44.0 (22.0)*</td>
<td>73.0 (20.0)*</td>
</tr>
<tr>
<td><strong>FEV₁/FVC</strong></td>
<td>83.0 (15.0)</td>
<td>80.1 (15.2)</td>
<td>31.4 (16.6)*</td>
</tr>
<tr>
<td><strong>PaO₂ (kPa)</strong></td>
<td>-</td>
<td>9.1 (1.13)</td>
<td>7.49 (1.71)</td>
</tr>
<tr>
<td><strong>PaCO₂ (kPa)</strong></td>
<td>-</td>
<td>5.73 (1.29)</td>
<td>6.64 (0.88)</td>
</tr>
<tr>
<td><strong>6MW (m)</strong></td>
<td>-</td>
<td>300 (199)</td>
<td>155 (180)</td>
</tr>
<tr>
<td><strong>QMVC (kg)</strong></td>
<td>46.0 (17.0)</td>
<td>21.5 (21.0)*</td>
<td>31.5 (11.0) *</td>
</tr>
<tr>
<td><strong>QMVC as a % of BMI</strong></td>
<td>172 (48.4)</td>
<td>106 (33.0) *</td>
<td>115 (21.1) *</td>
</tr>
<tr>
<td><strong>TwQ (kg)</strong></td>
<td>9.65 (3.0)</td>
<td>5.9 (3.3)*</td>
<td>6.5 (4.3)</td>
</tr>
</tbody>
</table>

BMI – body mass index, FFMI – fat free mass index, FEV₁ – forced expiratory volume in one second, FVC – functional vital capacity, 6MW – six minute walk distance, QMVC – quadriceps maximal voluntary contraction, TwQ – Quadriceps twitch force

*p<0.05 or smaller between patient group and control group

¥p<0.05 or smaller between restrictive and COPD group
<table>
<thead>
<tr>
<th></th>
<th>Control Group</th>
<th>Restrictive Group</th>
<th>COPD Group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Median (IQR)</td>
<td>Median (IQR)</td>
<td>Median (IQR)</td>
</tr>
<tr>
<td>MyHC I %</td>
<td>47.7 (9.3)</td>
<td>35.3 (18.5) ¥*</td>
<td>22.1 (16.3)*</td>
</tr>
<tr>
<td>MyHC IIA %</td>
<td>37.0 (3.5)</td>
<td>37.4 (12.8) ¥</td>
<td>50.8 (10.0)*</td>
</tr>
<tr>
<td>MyHC IIX %</td>
<td>11.3 (13.0)</td>
<td>26.3 (15.5) *</td>
<td>24.8 (13.4)</td>
</tr>
<tr>
<td>Total Protein</td>
<td>3.82 (1.14)</td>
<td>5.71 (2.71) *</td>
<td>4.08 (0.55)</td>
</tr>
<tr>
<td>carbonylation</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>MDA – protein</td>
<td>0.46 (0.22)</td>
<td>1.02 (2.63)*</td>
<td>1.35 (0.58)*</td>
</tr>
<tr>
<td>adducts</td>
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<td></td>
</tr>
<tr>
<td>Protein tyrosine</td>
<td>0.24 (0.06)</td>
<td>0.76 (0.51)*</td>
<td>0.48 (0.30)*</td>
</tr>
<tr>
<td>nitration</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mn-SOD</td>
<td>0.70 (0.21)</td>
<td>0.96 (0.351) *</td>
<td>0.50 (0.165)</td>
</tr>
<tr>
<td>Catalase</td>
<td>1.32 (0.49)</td>
<td>1.54 (0.45)</td>
<td>1.34 (0.24)</td>
</tr>
</tbody>
</table>

MyHC - myosin heavy chain isoform, CS - citrate synthase, PFK - phosphofructokinase, HAD-hydroxyacyl-CoA dehydrogenase, GlyP - glycogen phosphorylase, MDA - malondialdehyde, Mn-SOD - manganese superoxide dismutase. Optical densities of the oxidative stress markers in all cases were expressed as the ratio of the optical densities of the specific antigen to those of GAPDH. The number of subjects for which biopsy data are available may be obtained by examination of figures of 2 & 3.

* p<0.05 or smaller between patient group and control group

¥p<0.05 or smaller between restrictive and COPD group
**Figure 1:** Quadriceps strength in the 3 patient groups measured as Maximal Voluntary Contraction Force (Panel A) and unpotentiated quadriceps twitch tension (Panel B). Quadriceps strength was reduced in both scoliosis and COPD patients compared with controls judged by MVC (p=0.02 and p=0.04 respectively) and, in the case of restrictive patients, by TwQ (p=0.013).

**Figure 2:** Myosin Heavy chain expression in the three groups is shown; upper panel type 1, middle panel type IIa and lower panel type IIx. Scoliosis patients differed from controls in Type I Myosin heavy chain isoform expression, p=0.028 and Type IIx p=0.01. COPD patients differed from controls in expression of type I and IIa myosins (p=0.009 and p=0.0009 respectively).

**Figure 3** Protein tyrosine nitration (upper panel) and MDA protein adducts (lower panel) in all 3 groups. In scoliosis patients MDA-protein adducts (p=0.002), protein tyrosine nitration (p=0.001) were both increased. These variables were also increased in COPD patients, p=0.002 and p=0.008, respectively.

**Figure 4** Representative examples of MDA-protein adducts and GAPDH in quadriceps of control subjects and both severe scoliosis and COPD patients. Several MDA-protein adducts were detected. Monoclonal anti-GAPDH antibody was used to control equal loading among various lanes.
**Figure 5**  Representative examples of protein tyrosine nitration (total 3-nitrotyrosine immunoreactivity) and GAPDH in quadriceps of control subjects and both severe scoliosis and COPD patients. Several tyrosine nitrated proteins were detected. Monoclonal anti-GAPDH antibody was used to control equal loading among various lanes.
Figure 1

A) MVC

B) TwQ
Figure 2.

**MyHC I**

![MyHC I graph](image1)

**MyHC IIa**

![MyHC IIa graph](image2)

**MyHC IIx**

![MyHC IIx graph](image3)
Figure 3.

Protein tyrosine nitration

MDA protein adducts
Figure 4

MDA-protein adducts

MW (kDa)

Controls  Scoliosis patients  COPD patients
Figure 5

Protein tyrosine nitration

MW (kDa)

Controls  Scoliosis patients  COPD patients
References


