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Glutathione and glutamate levels in the diaphragm of patients with chronic obstructive pulmonary disease

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ABSTRACT: Recently, decreased glutamate (Glu) and reduced glutathione (GSH) levels were reported in the quadriceps femoris of patients with chronic obstructive pulmonary disease (COPD). The aim of the present study was to investigate whether Glu and GSH levels are also modified in the diaphragm of these patients.

Nine male COPD patients (forced expiratory volume in one second (FEV_1) range 28–68% of the predicted value) and seven male patients with normal pulmonary function (mean±SD FEV_1 86±3% pred) submitted to thoracotomy were included. Biopsy specimens were taken from the diaphragm (both groups) and the quadriceps femoris (COPD group alone) in order to assess fibre size, myosin heavy chain expression, GSH levels and amino acid profile.

The COPD group was characterised by preserved fibre size, a higher proportion of type I fibres (mean±SEM 70±3 *versus* 26±4%), and higher Glu and GSH content in the diaphragm compared to the quadriceps muscle. However, Glu and GSH levels were similar in diaphragm from the COPD and control groups. Glu level correlated with GSH level in both muscles. No significant correlation was found between Glu or GSH level and fibre size or proportions.

This study shows that glutamate and reduced glutathione levels are preserved in the diaphragm of chronic obstructive pulmonary disease patients. Alterations in glutamate and reduced glutathione metabolism are muscle-specific in chronic obstructive pulmonary disease, affecting the quadriceps femoris but not the diaphragm. Glutamate and reduced glutathione levels are strongly interrelated in both muscles, independent of fibre type distribution and fibre size.
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Recent studies have reported severely decreased levels of the amino acid glutamate (Glu) in lower limb muscles of patients with chronic obstructive pulmonary disease (COPD). This reduction in Glu levels was found in both a random (mixed) COPD group [1, 2] and a homogenous group of COPD patients with emphysema [3]. The reduced Glu concentration was found to be independent of the limb muscle group studied, since a reduction in Glu concentration was found both in the tibialis anterior and quadriceps femoris [1, 2].

Further insight into the underlying causes and mechanisms of muscle Glu depletion is of importance as Glu is involved in many metabolic pathways both at rest and during exercise [4]. Glu is an important precursor for the first and rate-limiting step in the synthesis of reduced glutathione (GSH), one of the most important antioxidants in muscle. A recent study revealed reduced levels of muscle Glu and GSH in COPD patients, and a high correlation between Glu and GSH levels in both patients and controls [3]. Reduced muscle GSH levels could result in an antioxidant/oxidant imbalance, increasing the susceptibility of the muscle to oxidative stress and cell injury [5]. Avoiding intracellular Glu depletion may therefore be of clinical importance in the prevention of oxidative stress-induced muscle damage and muscle dysfunction in patients with COPD. To date, it is not known whether the reduction in Glu and GSH levels in COPD is limited to the skeletal muscle compartment or is a generalised phenomenon affecting all striated muscles, including the diaphragm, of these patients. In addition, to the present authors' knowledge, no studies have ever reported on the amino acid concentrations of human diaphragm in healthy conditions or in COPD.

In COPD patients, an elevated proportion of myosin heavy chain (MyHC) type I (MyHC-I) fibres has been found in the diaphragm [6], whereas a decreased proportion of MyHC-I fibres has been found in peripheral skeletal muscle [7–10]. Two studies have examined the relationship between muscle amino acid concentrations and fibre type proportions. Results with respect to Glu were strikingly different between the studies. Whereas lower concentrations of Glu were found in fast-twitch rat muscle, higher concentrations were found in a separate pool of MyHC type II (MyHC-II) fibres in human vastus lateralis [11, 12]. Therefore, the possible existence of different Glu and GSH levels in the diaphragm and quadriceps femoris of COPD patients, and the potential association with differences in fibre type, must be addressed.

It is hypothesised that a reduction in Glu and GSH levels in patients with COPD affects all striated muscles including the diaphragm, and is unrelated to changes in fibre type distribution. Therefore, in COPD patients and patients with

normal pulmonary function submitted to thoracotomy, biopsy specimens were taken from the diaphragm and, in the COPD group, also from the quadriceps femoris in order to assess MyHC expression, GSH levels and amino acid profile.

Methods

Study population

Sixteen male patients (aged 62±10 yrs) undergoing thoracotomy for localised lung neoplasm were selected for the study. The patients were systematically selected through the Lung Cancer Multidisciplinary Committee and Dept of Surgery, Hospital del Mar, Barcelona, Spain. Nine of these patients were diagnosed with COPD, as determined from a clinical history consistent with chronic bronchitis and/or emphysema, a long history of cigarette smoking and pulmonary function test results consistent with irreversible airflow obstruction (forced expiratory volume in one second (FEV1) <80% of the predicted value and FEV1/FVC ratio <70% pred) according to European Respiratory Society criteria [13]. Exclusion criteria for both COPD and control patients included asthma, coronary disease, low body weight (body mass index (BMI) <20 $kg \cdot m^{-2}$), chronic metabolic diseases (*e.g.* diabetes, hypo- or hyperthyroidism), orthopaedic diseases, suspected paraneoplastic or myopathic syndromes, previous abdominal or thoracic surgery, and/or treatment with steroids, hormones or cancer chemotherapy. All patients were current smokers or had a history of previous cigarette smoking (cumulative smoking index of 59±28 *versus* 59±27 packs-yrs in COPD *versus* control patients). The study was conducted in accordance with World Medical Association guidelines for research in humans [14]. An institutional ethics board approved the protocols.

Pulmonary and respiratory muscle function tests

Forced spirometry, inspiratory capacity (Datospir 92; SIBEL, Barcelona, Spain) and thoracic gas volume (Masterlab; Jaeger, Würzburg, Germany) measurements were made in each patient and compared to reference values from a Mediterranean population [15, 16]. While patients breathed through a two-way valve (Hans Rudolph, Kansas City, MO, USA), maximal inspiratory oesophageal and transdiaphragmatic pressure during a maximal sniff manoeuvre at functional residual capacity were measured [17]. Maximal inspiratory pressure was measured using a manometer (BP1050; Biopac Systems, Goleta, CA, USA) with an occludable mouthpiece (SIBELMED, Sibel, Spain) according to the techniques described by BLACK and HYATT [18] and compared to the values of WILSON *et al.* [19]. Arterial blood samples were analysed for arterial oxygen (P_aO_2) and carbon dioxide tension (P_aCO_2) and pH (ABL 330; Radiometer, Copenhagen, Denmark). In addition, body weight was measured using an electronic beam scale with a digital readout to the nearest 0.1 kg with subjects standing barefoot and wearing light indoor clothing. Body height was measured to the nearest 0.1 cm and BMI (weight in kilograms divided by height in metres squared) calculated.

Muscle biopsy

In all COPD and control patients, biopsy specimens (~0.5 cm×0.5 cm×0.5 cm) were obtained from the costal diaphragm at the beginning of the surgical procedure. In addition, in the COPD group, biopsy specimens were synchronously taken from the vastus lateralis. Diaphragm samples were taken from the apposition zone, ~2 cm from the costodiaphragmatic angle at the mid-axillary line. Samples from the middle portion of the vastus lateralis were considered to be representative of the quadriceps muscle. Each biopsy specimen was divided into two pieces and these pieces randomly used for histomorphometric and biochemical analyses. The biopsy specimens were quick-frozen in isopentane, cooled in liquid nitrogen and stored at -70°C until required for analysis.

Histomorphometry

Sections (10 μm) of one piece of the muscle sample were obtained by adjusting the holder (in increments of 5°) until the minimum cross-sectional area (CSA) was obtained, which was defined as truly transverse for MyHC-I (CSA-I) and -II fibres (CSA-II) [20, 21]. Cross-sections were processed for immunohistochemical procedures [21]. CSA, mean least diameter and proportions of MyHC-I and -II fibres were assessed using a light microscope (Olympus, Series BX50F3; Olympus Optical Co., Tokyo, Japan) coupled to an image-digitising camera (Pixera studio, Version 1.2; Pixera Corporation, Los Gatos, CA, USA) using image analysis software (NIH Image, Version 1.60; Frederick, MD, USA). At least 100 fibres were measured per diaphragm and vastus lateralis biopsy specimen (mean number of fibres analysed 316±43) [22, 23]. MyHC-I and -II monoclonal antibodies were used to identify MyHC-I and -II fibres (MHCs and MHCf clones; Biogenesis, Poole, UK) as described elsewhere [24].

Muscle amino acid profile

The remaining piece of frozen muscle tissue was deproteinised with sulphosalicylic acid for determination of amino acid concentrations. After adding glass beads (1 mm), the muscle tissue was homogenised using a Mini-beater (Biospec Products, Bartlesville, OK, USA). The amino acid profiles of the diaphragm and vastus lateralis were analysed in the same batch run by fully automated high-performance liquid chromatography (HPLC) [25, 26]. Levels of the following amino acids were measured: glutamine, glycine, alanine, valine, isoleucine, leucine, phenylalanine, tyrosine, arginine, histidine, lysine, methionine, threonine, tryptophan, alpha-aminobutyric acid, asparagine, citrulline, Glu, ornithine, serine, and taurine. The GSH concentration of the muscle specimen was assessed in the same HPLC run. The branched-chain amino acid concentration was calculated by summing the leucine, isoleucine and valine concentrations. The total amino acid concentration was also calculated.

Statistical analysis

Results are expressed as mean±SD, and, in figures, amino acid and fibre type measurements are presented as mean±SEM. COPD and control group data were compared using an unpaired t-test. In order to assess whether muscle determination differences between the vastus lateralis and diaphragm in the COPD group were significant, a paired t-test (or Wilcoxon signed-rank test in the case of abnormal distribution) was used. A two-tailed probability value of <0.05 was considered significant. The relationships between muscle amino acid concentrations, GSH and fibre type distribution within each

muscle group were studied by calculating Pearson's correlation coefficient (or Spearman's nonparametric correlation coefficient in the case of unequal variances).

Results

Nine patients with COPD (FEV1 52±4% pred, range 28–68% pred) and seven controls (FEV1 86±3% pred) participated in the study (table 1). Age, height, body weight and BMI did not differ between the two groups. The COPD group was characterised by lower FVCs ($p<0.01$) and higher residual volumes ($p<0.05$) than the control group. P_{a,O_2} was lower in the COPD group than in the control group (8.99±1.08 *versus* 10.9±1.85 kPa (67.6±8.1 *versus* 81.7±13.9 mmHg), $p<0.05$). P_{a,CO_2} was higher in the COPD group (5.49±0.36 *versus* 4.81±0.36 kPa (41.3±2.7 *versus* 36.2±2.7 mmHg), $p<0.01$). No difference was found in pH (7.43±0.03 *versus* 7.42±0.01).

COPD group versus *controls*

No significant difference was found in the proportion of MyHC-I in the diaphragm between the COPD and control groups (table 1). Also, no difference was found in CSA-I and -II in the diaphragm between the COPD and control groups.

No difference was found in Glu (fig. 1a) and GSH concentration (fig. 1b) in the diaphragm between the COPD and control groups. In addition, the total amino acid concentration measured in the diaphragm did not differ between the control and COPD groups (fig. 1c).

Diaphragm versus *quadriceps femoris*

The COPD patients showed a higher proportion of MyHC-I in the diaphragm than in the quadriceps femoris (70 *versus* 26%, $p<0.001$), and thus a lower proportion of MyHC-II (table 2). In the COPD group, CSA-I and -II did not differ between the diaphragm and quadriceps femoris.

Glu (fig. 1a) and GSH (fig. 1b) levels were significantly lower in the quadriceps femoris than in the diaphragm ($p<0.05$). The amino acid profiles of the diaphragm and quadriceps femoris are shown in table 3. Total amino acid concentration showed no difference between the two muscles (fig. 1c). Besides Glu, the levels of several other amino acids in the COPD group were lower in the quadriceps femoris than in the diaphragm. Furthermore, the Glu/GSH ratio was lower in the quadriceps femoris ($p<0.05$). However, no difference was observed for glutamine and taurine, amino acids that are generally present in very high concentrations in skeletal muscles. This may explain why there was no difference in the total amino acid concentration between the quadriceps femoris and the diaphragm in the COPD group.

Table 1. – General characteristics of study population

	Controls	COPD group
Subjects n	7	9
Age yrs	65±3	68±2
Height cm	168±3	167±2
Weight kg	75.3±4.2	73.4±4.3
BMI $kg \cdot m^{-2}$	26.5±1.0	26.3±1.3
FEV1 % pred	86±3	52±4***
FVC % pred	84±2	61±5**
TLC % pred	91±3	102±7
RV % pred	100±9	155±21*
ITGV % pred	102±7	129±17
$P_{I,max}$ % pred	82±22	86±26
$P_{oes,max}$ cmH_2O	-76±14	-67±22
$P_{di,max}$ cmH_2O	99±19	88±58

Data are presented as mean±SD. COPD: chronic obstructive pulmonary disease; BMI: body mass index; FEV1: forced expiratory volume in one second; FVC: forced vital capacity; TLC: total lung capacity; RV: residual volume; ITGV: intrathoracic gas volume; $P_{I,max}$: maximal inspiratory pressure measured at the mouth (Müller manoeuvre); $P_{oes,max}$: maximal oesophageal pressure during sniff manoeuvre; $P_{di,max}$: maximal transdiaphragmatic pressure during sniff manoeuvre; % pred: percentage of the predicted value. *: $p<0.05$; **: $p<0.01$; ***: $p<0.001$ *versus* controls.

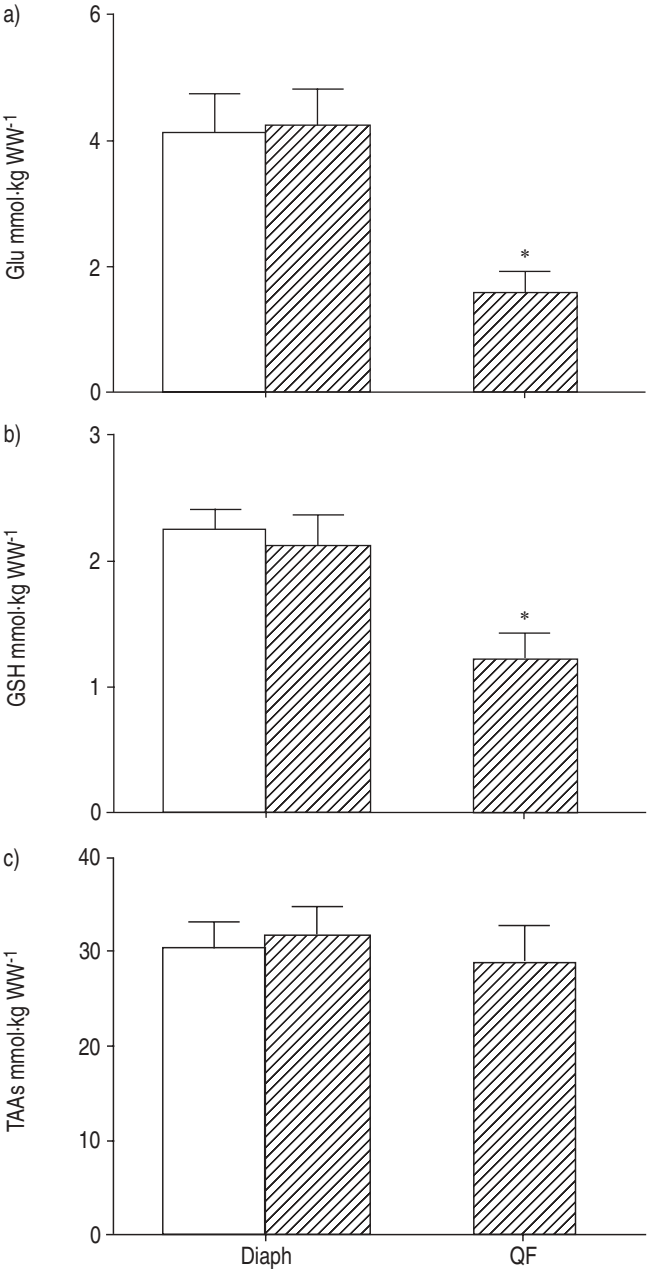

Fig. 1. – Concentration of: a) glutamate (Glu); b) reduced glutathione (GSH); and c) total amino acids (TAAs) in the diaphragm (diaph) and quadriceps femoris (QF) of chronic obstructive pulmonary disease (COPD) patients (▨) and control subjects (□). Data are presented as mean±SEM. WW: wet weight. *: $p<0.05$ *versus* diaphragm in COPD.

Table 2. – Size and distribution of fibre types in the diaphragm and quadriceps femoris[#]

	Control diaphragm	COPD group	
		Diaphragm	Vastus lateralis
CSA-I μm^2	3015±198	3534±358	3610±459
CSA-II μm^2	3421±269	3994±369	3837±719
MyHC-I %	68.8±4.0	70.2±3.0	26.4±3.7***
MyHC-II %	35.6±6.4	29.8±3.0	74.5±3.7***

Data are presented as mean±SEM. COPD: chronic obstructive pulmonary disease; MyHC-I and -II: myosin heavy chain type I and type II; CSA-I and -II: cross-sectional area of MyHC-I and -II fibres, respectively. [#]: vastus lateralis. ***: p<0.001 *versus* COPD group diaphragm.

Correlations

A highly significant correlation was found between muscle Glu and GSH levels (fig. 2c) in the diaphragm (r=0.84, p<0.01) and quadriceps femoris (r=0.88, p<0.01). However, no significant relationship was found between intramuscular Glu or GSH level and fibre type distribution in either diaphragm (Glu r=-0.01; GSH r=-0.26, NS) or quadriceps femoris (Glu r=0.32; GSH r=0.29, NS) (fig. 2a and 2b). Total amino acid concentration was neither related to fibre type distribution nor CSA-I and -II in both muscles (data not shown). No relationship was evident between Glu or GSH levels and muscle fibre size (CSA-I or -II). Finally, levels of GSH and Glu (or other amino acids) of both the diaphragm and quadriceps femoris were not related to body weight or BMI (data not shown).

Discussion

This is the first study demonstrating that diaphragmatic levels of Glu and GSH do not differ between COPD patients and individuals with normal pulmonary function. Although it has previously been demonstrated that levels of Glu and GSH are reduced in the peripheral muscles of COPD patients, the present study demonstrates that such amino acid disturbance is not a generalised (systemic) phenomenon, suggesting that the diaphragm discloses amino acid adaptation in the face of the disease.

At first sight, it is surprising that the diaphragm, but not skeletal muscles, of COPD patients is able to preserve normal Glu and GSH levels. Respiratory and peripheral muscles are exposed to numerous factors that can negatively influence amino acid levels (malnutrition, inflammation, chronic hypoxaemia, drugs, *etc.*). However, the difference could be related to the fact that diaphragmatic myofibres are continuously working against the increased mechanical overload imposed by persistent airflow obstruction and pulmonary hyperinflation. This increased activity goes on for many years, and, therefore, structural changes can represent chronic diaphragmatic adaptations. If the mechanical loading imposed by the disease is able to emulate a training effect, preserved Glu and GSH levels could be considered adaptive metabolic changes within diaphragm cells. Indeed, peripheral muscle training has been shown to increase GSH and Glu content in healthy subjects [4, 27].

A discrepancy may arise when comparing the chronic mechanical loading in the stable to that in the acute (exercise or exacerbation) state. It is well known that acute bouts of mechanical stress and factors such as free radicals, oxidation and increased protease activity are able to induce or

Table 3. – Free amino acid pool and glutathione (GSH) levels in the diaphragm and quadriceps femoris[#]

	Control diaphragm	COPD group	
		Diaphragm	Vastus lateralis
Glu $\mu mol \cdot kg\ WW^{-1}$	4161±588	4253±558	1584±333*
GSH $\mu mol \cdot kg\ WW^{-1}$	2257±160	2140±228	1226±201*
Glu/GSH	1.8±1.5	2.0±0.5	1.3±0.1*
Asn $\mu mol \cdot kg\ WW^{-1}$	282±8	280±32	157±23*
Ser $\mu mol \cdot kg\ WW^{-1}$	650±102	748±104	451±79*
Gln $\mu mol \cdot kg\ WW^{-1}$	9328±898	8808±1132	9097±1306
Gly $\mu mol \cdot kg\ WW^{-1}$	1025±54	966±78	819±122
Thr $\mu mol \cdot kg\ WW^{-1}$	760±50	812±91	540±79*
His $\mu mol \cdot kg\ WW^{-1}$	394±49	405±51	339±59
Cit $\mu mol \cdot kg\ WW^{-1}$	87±17	56±9	106±26*
Ala $\mu mol \cdot kg\ WW^{-1}$	2388±246	2065±184	1327±240**
Tau $\mu mol \cdot kg\ WW^{-1}$	5835±697	7031±759	7332±1544
Arg $\mu mol \cdot kg\ WW^{-1}$	442±66	468±56	375±90
α-ABA $\mu mol \cdot kg\ WW^{-1}$	56±7	45±4	37±6
Tyr $\mu mol \cdot kg\ WW^{-1}$	83±8	99±9	74±11[+]
Val $\mu mol \cdot kg\ WW^{-1}$	341±35	349±38	271±32
Met $\mu mol \cdot kg\ WW^{-1}$	73±19	83±20	55±21
Ile $\mu mol \cdot kg\ WW^{-1}$	116±13	118±13	84±10
Phe $\mu mol \cdot kg\ WW^{-1}$	149±27	124±14	80±12[¶]
Trp $\mu mol \cdot kg\ WW^{-1}$	324±49	315±80	184±35
Leu $\mu mol \cdot kg\ WW^{-1}$	208±24	184±14	140±19*
Orn $\mu mol \cdot kg\ WW^{-1}$	154±18	155±19	163±44
Lys $\mu mol \cdot kg\ WW^{-1}$	693±75	690±95	694±176
BCAAs $\mu mol \cdot kg\ WW^{-1}$	633±65	651±60	493±66
Total AAs $\mu mol \cdot kg\ WW^{-1}$	30467±3049	31729±3091	28836±4388

Data are presented as mean±SEM. COPD: chronic obstructive pulmonary disease; Glu: glutamate; GSH: reduced glutathione; Asn: asparagines; Ser: serine; Gln: glutamine; Gly: glycine; Thr: threonine; His: histidine; Cit: citrulline; Ala: alanine; Tau: taurine; Arg: arginine; α-ABA: alpha-aminobutyric acid; Tyr: tyrosine; Val: valine; Met: methionine; Ile: isoleucine; Phe: phenylalanine; Trp: tryptophan; Leu: leucine; Orn: ornithine; Lys: lysine; BCAA: branched-chain amino acid (AA); WW: wet weight. [#]: vastus lateralis. [¶]: p=0.08; [+]: p=0.07; *: p<0.05; **: p<0.01 *versus* COPD group diaphragm.

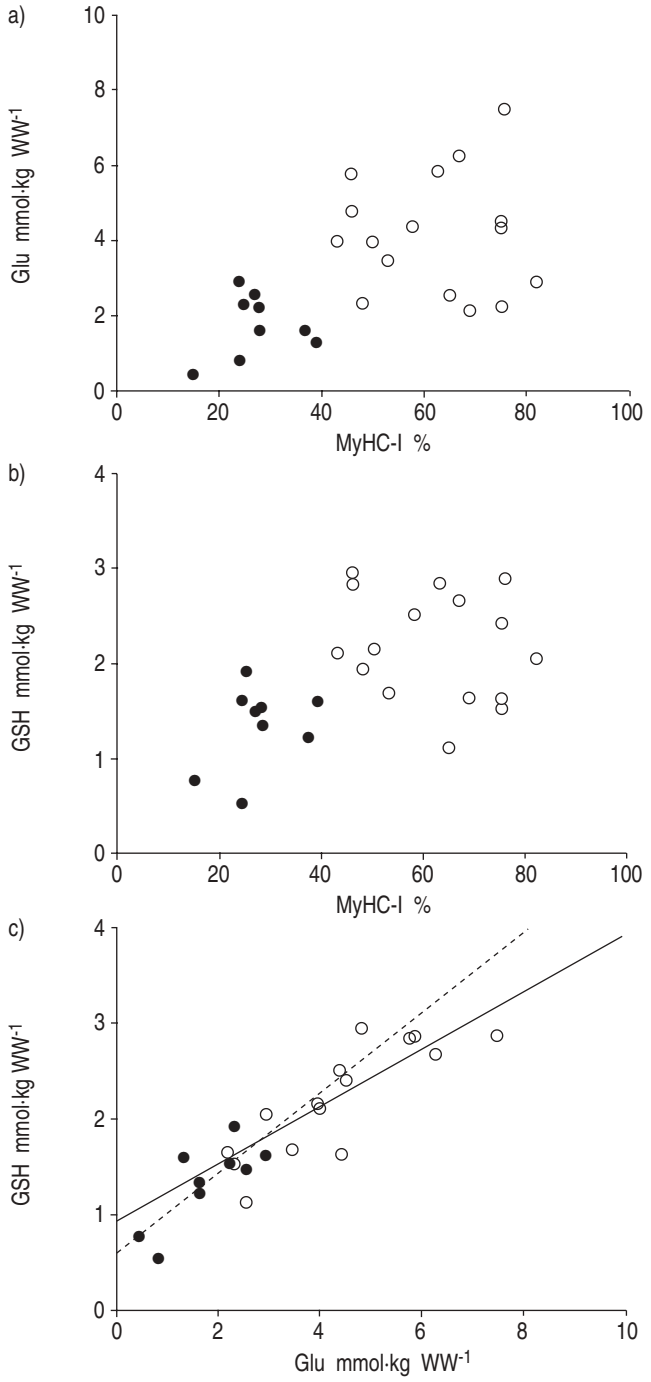

Fig. 2. – Scatter plots showing correlation between: a) proportion of myosin heavy chain type I (MyHC-I) fibres and muscle glutamate (Glu) levels; b) proportion of MyHC-I fibres and muscle reduced glutathione (GSH) levels; and c) muscle Glu and GSH levels in quadriceps femoris (●) and diaphragm (○). Regression lines for quadriceps femoris (- - - -) and diaphragm (——) are shown in c). WW: wet weight.

accentuate diaphragm injury [28–30]. There is evidence for increased systemic oxidative stress in COPD patients during exercise, and, perhaps, even at rest [31, 32], indicating the importance of optimisation of muscle antioxidant levels in COPD. Some recent evidence permits the suggestion to be made that the present findings showing a preserved level of Glu and GSH in the diaphragm could be suboptimal in the setting of severe COPD. Indeed, increased susceptibility to diaphragm injury has been found in COPD patients submitted to acute inspiratory loading [5]. It was recently shown that the diaphragm of COPD patients exhibits up to 300% greater susceptibility to injury (as assessed by sarcomere disruption) compared to that of control subjects [5]. Besides a negative morphological effect, an acute bout of exercise is also known to negatively influence muscle amino acid metabolism in COPD. Recently, it was shown that 20 min of moderate-intensity exercise resulted in depletion of the skeletal muscle amino acid pool, including that of Glu [1]. Further research is needed to test whether this also holds for acute respiratory muscle loading.

Glutamate concentration in relation to fibre type

The high proportion of MyHC-I fibres present in the diaphragm of the COPD group (70%) and low proportion of MyHC-I in the quadriceps femoris (26%) were in line with earlier data [6, 7, 10]. There is a IIb/x to I shift towards more oxidative metabolism in the diaphragm, and a I to IIb/x shift towards less oxidative metabolism in the lower limb muscle of patients with COPD. To date, two studies examining the relationship between amino acid profile and fibre typing of muscle have been conducted [11, 12]. In rats, fast-twitch muscles (plantaris and gastrocnemius) show lower levels of a few amino acids (including Glu) but higher levels of other amino acids compared to slow-twitch muscles (soleus) [11]. Different results were obtained in a recent study in which the amino acid profile was examined in separate pools of different (single) fibre types of the vastus lateralis muscle of five endurance-trained humans [12]. This study showed that the Glu concentration is 9% higher in MyHC-II than in MyHC-I fibres, whereas comparable values can be found for most of the remaining amino acids.

In the present study, Glu levels were 63% higher in the diaphragm (30% MyHC-II fibres) than in the quadriceps femoris (74% MyHC-II fibres) of COPD patients. This suggests that the differences in Glu concentration are likely to be >63% in the separate pools of fibre types in COPD. In addition, no significant relationship was found between levels of Glu or total amino acids and proportions of MyHC fibres in both quadriceps femoris and diaphragm. These findings demonstrate that the low proportion of MyHC-I fibres is not responsible for the reduced Glu level which has consistently been observed in the peripheral skeletal muscle of patients with COPD [1, 3, 33]. Factors other than fibre type distribution play a significant role in the observed differences in Glu levels (and total amino acid profile) between the two muscle groups in COPD.

Glutathione concentration in relation to fibre type

In the present study, the absolute GSH concentration was 42% higher in the diaphragm than in the quadriceps femoris of patients with COPD. However, no significant relationship was found between GSH levels and the proportion of MyHC-I. This suggests that other factors, in addition to a reduced oxidative capacity, are responsible for the observed reduction in GSH concentration in the peripheral muscles in COPD. RABINOVICH *et al.* [27] recently showed that endurance training did not increase GSH levels in the peripheral muscle of patients with COPD. The present data are in line with their findings, suggesting that enhancement of the oxidative capacity of muscle may not be fully successful in protecting against oxidative stress in COPD patients.

The proportion of MyHC-I fibres in the non-COPD group

is somewhat higher than those previously reported by NGUYEN *et al.* [34] and LEVINE and coworkers [6, 35] (45 and 41%). This may be related to differences in the criteria used for selection of a control group. In the present study, it was decided to include subjects with localised lung neoplasm. The possibility that the presence of lung cancer contributes to the slightly higher level of MyHC-I fibres in the diaphragm cannot be totally excluded, although the exact underlying mechanism is difficult to elucidate. NGUYEN *et al.* [34] and LEVINE and coworkers [6, 35] included subjects with mild pulmonary impairment undergoing resection of solitary pulmonary nodules and brain-dead organ donors. It is possible that both the underlying lung diseases in the first group and the amount of time in receipt of ventilatory support and the decreased central ventilatory drive due to brain death in the second group may be an important bias. Furthermore, sex differences between the studies may possibly contribute to the small differences in fibre typing between the control groups. Whereas the present study included only male patients, NGUYEN *et al.* [34] and LEVINE and coworkers [6, 35] conducted studies in which 70, 100 and 65% of the study population were female. More studies are needed to confirm whether sex indeed plays a role in fibre type distribution in diaphragm muscle.

Glutamate concentration in relation to glutathione concentration

To the present authors' knowledge, this is the first study reporting amino acid concentrations in the diaphragm of subjects with normal lung function and patients with COPD. Higher levels of Glu were found in the diaphragm compared to the quadriceps femoris of patients with COPD. The levels of several other amino acids (*i.e.* alanine, serine, threonine and leucine) were also higher in the diaphragm than in the quadriceps femoris. Moreover, the mean difference in total amino acid concentration (9%) was much smaller than that of Glu (63%). However, it is beyond the scope of the present article to discuss these differences further.

In the present study, Glu concentration was highly correlated with GSH concentration in quadriceps femoris, which is in agreement with previous data [3]. Moreover, in the diaphragm, a comparably high correlation coefficient was also found between Glu and GSH concentration. These data suggest that muscle Glu is an important precursor for the first and rate-limiting step in the synthesis of the antioxidant GSH, independent of the muscle group studied (diaphragm *versus* lower limb muscles).

Potential limitations of the present study

In the present study, neither the levels of amino acids nor the histomorphometric characteristics of the quadriceps femoris were assessed in the control volunteers. From a methodological point of view, it is acknowledged that inclusion of control values for Glu concentration in the quadriceps femoris could have been complementary data. However, the lack of such data does not affect the main aim or conclusions of the present study. The present results are consistent with other studies assessing amino acid levels in the peripheral muscles of COPD patients. Although the quadriceps femoris of the COPD group showed comparable levels of Glu to those observed in other settings, these levels were lower than those described in the quadriceps femoris of healthy controls [1, 3, 33]. It should also be mentioned that, although the histomorphological and biochemical analysis utilised individual segments of muscle, both of the two segments originated from the same biopsy specimen. Furthermore, the variation in the biochemical and histomorphometric analyses ranged 13–21 and 4–14%, respectively. These findings suggest a low likelihood that the muscle samples used for the distinctive analyses were not representative of each other. Finally, as the Glu and GSH concentrations were expressed per kilogram of wet weight, the data could be influenced by differences in water content between the study groups. In the present study, the water content of the muscle samples was not measured, but, instead, data from a previous study were reassessed [33]. No significant difference was found in water content between muscle samples from COPD patients (77.0±0.7%, n=31) and control volunteers (77.3±0.7%, n=26).

Conclusions

The present study illustrates that glutamate and reduced glutathione levels are preserved in the diaphragm of chronic obstructive pulmonary disease patients, suggesting that the diaphragm has the capacity to show adaptive metabolic changes and is relatively "protected" against disease-related disturbances in amino acid and thus protein metabolism. Disturbances in glutamate and reduced glutathione metabolism are muscle-specific in chronic obstructive pulmonary disease, affecting the quadriceps femoris but not the diaphragm. Glutamate and reduced glutathione levels are strongly interrelated in both muscles but independent of fibre type distribution and fibre size.

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