

# Muscle fiber type IIX atrophy is involved in the loss of fat-free mass in chronic obstructive pulmonary disease

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# Muscle fiber type IIX atrophy is involved in the loss of fat-free mass in chronic obstructive pulmonary disease<sup>1-3</sup>

Harry R Gosker, Mariëlle PKJ Engelen, Henk van Mameren, Paul J van Dijk, Ger J van der Vusse, Emiel FM Wouters, and Annemie MWJ Schols

## ABSTRACT

**Background:** Although the loss of peripheral muscle mass has been shown convincingly in chronic obstructive pulmonary disease (COPD), the underlying pathogenesis remains unclear.

**Objective:** The aim of the present study was to determine the relations between skeletal muscle fiber types, fiber cross-sectional area (CSA), enzyme activities, and fat-free mass (FFM) in patients with COPD and in control subjects.

**Design:** In 15 patients with COPD and 15 healthy, age-matched control subjects, FFM was determined by dual-energy X-ray absorptiometry and bioelectrical impedance analysis. In biopsy specimens from the vastus lateralis fiber types, fiber CSA and activities of cytochrome oxidase (EC 1.9.3.1), succinate dehydrogenase (EC 1.3.99.1), and glycogen phosphorylase (EC 2.4.1.1) were examined immunohistochemically and histochemically.

**Results:** Compared with control subjects, patients with COPD had less FFM (49 compared with 59 kg,  $P = 0.030$ ) and lower mean fiber CSA (3839 compared with 4647  $\mu\text{m}^2$ ,  $P = 0.037$ ). A strong correlation ( $r = 0.87$ ,  $P < 0.001$ ) was observed between the FFM measured by bioelectrical impedance analysis and mean fiber CSA in patients with COPD. Within fiber-type categories the mean CSA of only the IIA/IIX and IIX fiber types was lower in patients than in control subjects [3358 compared with 4428  $\mu\text{m}^2$  ( $P = 0.022$ ) and 2566 compared with 4248  $\mu\text{m}^2$  ( $P = 0.003$ ), respectively]. In COPD, 20% of the type IIX fibers lacked stainable activities of cytochrome oxidase, succinate dehydrogenase, and glycogen phosphorylase, and this proportion correlated negatively with type IIX fiber CSA ( $r = -0.65$ ,  $P = 0.012$ ).

**Conclusions:** Muscle fiber atrophy occurs in the vastus lateralis in patients with COPD and contributes to the loss of muscle mass in COPD. Atrophy is specific to fiber types IIA/IIX and IIX and is associated with a disturbed metabolic capacity. *Am J Clin Nutr* 2002;76:113-9.

**KEY WORDS** Skeletal muscle mass, fiber atrophy, chronic obstructive pulmonary disease, fiber types, energy metabolism, cross-sectional area

## INTRODUCTION

Skeletal muscle weakness is frequently observed in patients with chronic obstructive pulmonary disease (COPD) (1) and plays a pivotal role in exercise intolerance (2). Recently, we

showed that skeletal muscle weakness is associated with wasting of extremity fat-free mass (FFM) but not with airflow obstruction (3). It is therefore crucial to examine the muscular abnormalities that are potentially involved in muscle wasting.

Whole skeletal muscle cross-sectional area (CSA) is  $\approx 25\%$  lower in patients with COPD than in control subjects, as determined by computed tomography (1). Muscle fiber atrophy can lead directly to a decrease in whole muscle CSA (assuming that the total amount of fibers is constant). However, information on muscle fiber dimensions in COPD is relatively scarce. A lower mean fiber CSA was found in 20 patients with COPD than in 9 healthy control subjects (4). A similar result was found in 8 patients with COPD who had severe muscle weakness while being treated with corticosteroids (5). As an alternative to fiber CSA, the fiber cross-sectional diameter has been determined. With the use of this method, fiber atrophy was shown in patients with emphysema who were severely emaciated (6). In none of these studies, however, were relations between muscle mass or body composition and fiber CSA reported, although muscle fiber atrophy may very well play a crucial role in the loss of FFM in COPD. Potential factors that may lead to muscle fiber atrophy include malnutrition, physical inactivity, and the use of oral corticosteroids. Because these factors affect type I and type II muscle fibers differently (7-9), analyzing the CSA of individual fiber types may provide insight into the relative contribution of these factors. Furthermore, studying the metabolic profile of the muscle fibers will help to clarify the intracellular and subsequent functional consequences of atrophy.

The present study was designed to address the following questions. Is there a relation between FFM and fiber CSA? Do type-specific differences in fiber CSA exist between patients with COPD and healthy control subjects? Is the capacity for energy metabolism disturbed in the affected fibers? To answer these

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questions, 15 patients with COPD and 15 healthy control subjects were evaluated by means of body-composition assessment and a needle biopsy of the vastus lateralis. Fiber types were identified immunohistochemically and histochemically, and the mean CSA of the fibers was determined. In addition, fibers were stained for cytochrome oxidase (COX; EC 1.9.3.1) and succinate dehydrogenase (SDH; EC 1.3.99.1) activities as markers of oxidative energy metabolism and for glycogen phosphorylase (GlyP; EC 2.4.1.1) activity as a marker of glycolytic energy metabolism.

## SUBJECTS AND METHODS

### Study population

A group of 15 patients (12 men and 3 women) with moderate-to-severe airflow obstruction and 15 healthy, age-matched subjects (13 men and 2 women) was studied. All the patients had COPD, according to American Thoracic Society guidelines (10), and chronic airflow limitation, defined as measured forced expiratory volume in 1 s ( $FEV_1$ ) <70% of the reference  $FEV_1$ . Furthermore, all patients had irreversible obstructive airway disease (<10% improvement in  $FEV_1$  from the predicted baseline value after  $\beta_2$ -agonist inhalation). At the time of the study, the patients were clinically stable and had not suffered from a respiratory tract infection or an exacerbation of their disease for  $\leq 4$  wk before the study. Information concerning the patients' usage and dosage of oral corticosteroids was retrieved from available hospital files and from the referring physicians. Doses were expressed as hydrocortisone equivalents/d. At the time of the biopsy, 7 of the 15 patients with COPD were receiving a maintenance dose of prednisolone (ranging from 5 to 10 mg/d). Of the remaining 8 patients, 4 had received corticosteroids at some time before the biopsy and 4 had never used corticosteroids. Exclusion criteria were malignancy; cardiac failure; distal arteriopathy; recent surgery; severe endocrine, hepatic, or renal disorders; and the use of anticoagulant medication. Written informed consent was obtained from all participants, and the study was approved by the medical ethical committee of the University Hospital Maastricht (Maastricht, Netherlands).

### Pulmonary function tests

All patients and control subjects underwent spirometry to determine their  $FEV_1$  values; the highest value from  $\geq 3$  technically acceptable determinations was used. The residual volume and intrathoracic gas volume were measured by whole-body plethysmography, and the diffusion capacity for carbon monoxide was measured with the single-breath method (Masterlab; Jaeger, Wurzburg, Germany). All values obtained were related to a reference value and expressed as a percentage of the predicted value (11).

### Assessment of body composition

The subjects' body height was measured to the nearest 0.5 cm while they stood barefoot, and the subjects' body weight was measured to the nearest 0.1 kg while they wore light clothing and no shoes. Whole-body FFM, which consists of lean mass and bone mineral mass, was determined by dual-energy X-ray absorptiometry ( $FFM_{DXA}$ ) in which each subject was scanned on a DPX bone densitometer (Lunar Corp, Madison, WI) as done

previously (12). The FFM of patients was also determined by using single-frequency (50 kHz) bioelectrical impedance analysis (BIA) (Xitron Technologies Inc, San Diego) while subjects were in a supine position.  $FFM_{BIA}$  was calculated by using the disease-specific equation of Schols et al (13). To adjust for body surface, weight parameters (in kg) were divided by body height squared (in  $m^2$ ), resulting in the body mass index and FFM index (14).

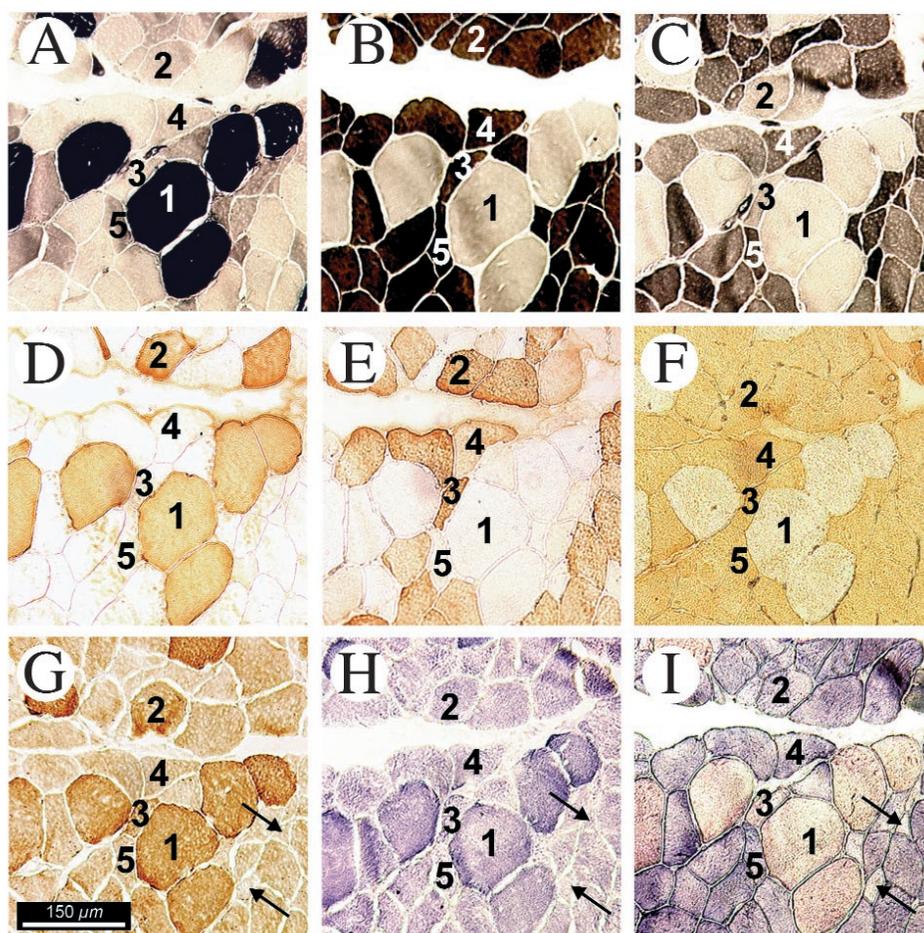
### Collection and processing of blood and muscle tissue

A blood sample was obtained by puncture of the radial artery while subjects breathed room air. This sample was used for the determination of arterial oxygen tension ( $PaO_2$ ) with the use of a blood gas analyzer (ABL 330; Radiometer, Copenhagen). Postabsorptive muscle biopsy specimens of the lateral part of the quadriceps femoris were obtained under local anesthesia by the needle biopsy technique (15). Biopsy specimens were placed in a drop of Tissue-tek (OCT compound; Sakura, Zoeterwoude, Netherlands) on a piece of cork, with the fiber orientation perpendicular to the plane of the cork. The specimens were frozen in melting isopentane precooled in liquid nitrogen and stored at  $-80^\circ C$ . Serial cryostat cross sections (10  $\mu m$ ) were made on a cryostat microtome at  $-20^\circ C$  and were mounted on slides, which were stored at  $-35^\circ C$  until analyzed. For each biopsy sample, 9 consecutive slides (each carrying 2 or 3 cross sections) were used for fiber-type characterization and enzyme activity staining. At least 100 (but up to 200) fibers coexisting on all 9 slides were numbered and analyzed as described earlier (16). Only fibers without artifacts that were part of a cluster of  $\geq 30$  fibers were included.

### Fiber type, metabolic enzyme characterization, and morphometry

The myofibrillar ATPase (EC 3.6.4.1) acidic and alkaline preincubations were performed at pH 4.4 (17) and 10.4 (18), respectively. Double preincubation was performed at pH 10.4 and 4.6 (19) with fixation of sections before the alkaline preincubation. After preincubation, sections were stained and mounted (20). Examples of myofibrillar ATPase staining are shown in **Figure 1**, A–C. Immunohistochemical analyses were performed with a panel of monoclonal antibodies (mAbs): anti-type I myosin heavy chain (MyHC) (mAb 219-1D1), anti-type IIA MyHC (mAb 333-7H1), and anti-types IIA + IIX MyHC (mAb 332-3D4) (21). Examples of MyHC staining are shown in **Figure 1**, D–F. The classification of fibers into fiber-type categories is shown in **Table 1**.

For COX activity staining, sections were incubated for 1 h in a 50 mmol tris:HCl/L buffer (pH 7.6) containing 0.22 mol sucrose/L, 14 mmol 3,3'-diaminobenzidine tetrahydrochloride/L, 80  $\mu$ mol cytochrome *c*/L, and 1300 U catalase (22). For SDH activity staining, sections were incubated for 1 h at  $37^\circ C$  in a 0.2 mol sodium phosphate/L buffer containing 0.1 mol succinic acid/L and 1.2 mmol nitro blue tetrazolium/L (23). For GlyP activity staining, sections were incubated for 5 min in a 43 mmol sodium acetate/L buffer (pH 5.6) containing 7 mmol glucose-1-phosphate/L, 1 mmol AMP/L, 0.01% (wt:vol) glycogen, and 15% (vol:vol) ethanol, after which the newly formed polysaccharide was colored with Lugol's iodine (23). COX and SDH cross sections were dehydrated in ethanol and mounted in Entellan (Merck, Darmstadt, Germany). GlyP sections were mounted in aqueous 15% glycerin and 15% gelatin (by wt). Three stain-



**FIGURE 1.** Example of histochemical analysis in 9 consecutive cross sections. Myofibrillar ATPase staining with acidic, alkaline, and double preincubation are shown in A–C, respectively (positive fibers are stained dark). Immunohistochemical analyses with anti–type I, anti–type IIA, and anti–types IIA + IIX monoclonal antibodies are shown in D–F, respectively (positive fibers are stained dark). Fibers numbered 1–5 are assigned to fiber types I, I/IIA, IIA, IIA/IIX, and IIX, respectively (staining intensities are given in Table 1). Cytochrome oxidase activity staining is shown in G (positive fibers are colored brown). Succinate dehydrogenase activity is shown in H (sites of glycogen phosphorylase activity are colored blue). Glycogen phosphorylase activity is shown in I (positive fibers are colored purple and negative fibers yellow). Arrows indicate CSG (cytochrome oxidase, succinate dehydrogenase, and glycogen phosphorylase)–negative fibers (G–I).

ing intensities were decided upon as reflecting fiber enzyme activity: positive (+), negative (–), or intermediate (±). Examples of enzyme activity staining are shown in Figure 1, G–I. Fibers lacking any stainable enzyme activity are referred to as CSG (COX, SDH, and GlyP)–negative fibers.

Fiber CSA was measured with an interactive image analysis system (Leica QWin Image Analysis System; Leica Microsystems BV, Rijswijk, Netherlands). The proportion of minifibers (MFs) was defined as the percentage of fibers having a CSA less than the mean CSA of the control group minus twice

**TABLE 1**  
Fiber type classification<sup>1</sup>

pH 4.4	mATPase		MyHC I	MyHC		Fiber type
	pH 10.4	Double incubation		MyHC IIA	MyHC IIX	
++	–	–	+	+/–	+/–	I
++	+/++	–	+	+	+	I/IIA
+/++	++	–	+	+	+	IIA
–	++	–	+/–	+	+	IIA/IIX
–/+	++	+/++	–	+	+	IIA/IIX
+	++	+/++	–	–	+	IIX

<sup>1</sup>mATPase, myofibrillar ATPase; MyHC, myosin heavy chain. Fibers were classified first on the basis of mATPase staining intensities (–, +, or ++) and thereafter by immunoreactivity (– or +). On the basis of these classifications, the following fiber-type categories were made: types I, I/IIA, IIA, IIA/IIX, IIX, and miscellaneous.

**TABLE 2**  
Subject characteristics and lung function data<sup>1</sup>

	Control subjects (n = 2 F, 13 M)	Patients with COPD (n = 3 F, 12 M)
Age (y)	64 ± 3 <sup>2</sup>	67 ± 9
BMI (kg/m <sup>2</sup> )	26.7 ± 3.0	23.9 ± 4.0 <sup>3</sup>
FFMI (kg/m <sup>2</sup> )	19.7 ± 2.2	17.2 ± 1.7 <sup>4</sup>
FEV <sub>1</sub> (% of predicted)	108 ± 18	42 ± 14 <sup>5</sup>
PaO <sub>2</sub> (kPa)	11.8 ± 1.7	9.9 ± 1.2 <sup>4</sup>
DL <sub>CO</sub> (% of predicted)	121 ± 23	63 ± 24 <sup>5</sup>
RV (% of predicted)	114 ± 15	167 ± 32 <sup>5</sup>
ITGV (% of predicted)	107 ± 18	144 ± 22 <sup>5</sup>

<sup>1</sup>FFMI, fat-free mass index; PaO<sub>2</sub>, arterial oxygen tension; DL<sub>CO</sub>, diffusion capacity for carbon monoxide; FEV<sub>1</sub>, forced expiratory volume in 1 s; RV, residual volume; ITGV, intrathoracic gas volume.

<sup>2</sup> $\bar{x} \pm SD$ .

<sup>3-5</sup>Significantly different from control subjects: <sup>3</sup> $P < 0.05$ , <sup>4</sup> $P < 0.01$ , <sup>5</sup> $P < 0.001$ .

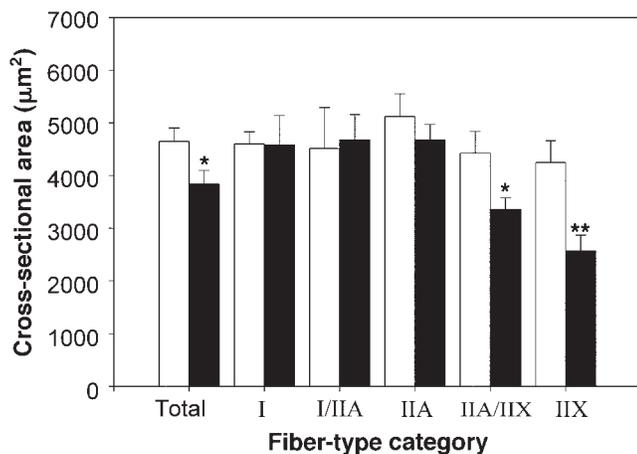
the SD. To avoid the influence of comparing the CSA of different fiber types, the proportion of minifibers was first calculated within each fiber-type category and was then recalculated as an overall percentage.

### Statistical analysis

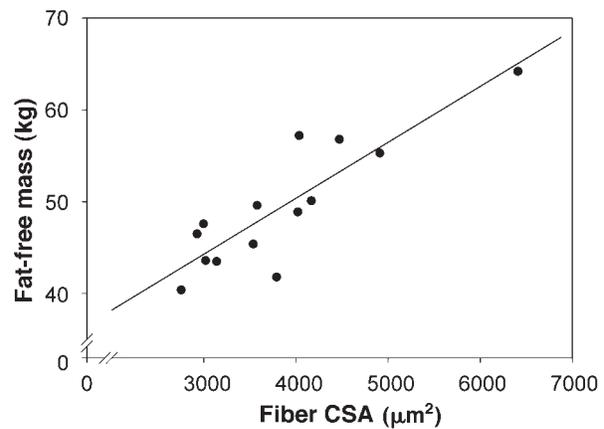
Data were analyzed with the unpaired Student's *t* test (corrected for unequal variances if appropriate), one-way analysis of variance (with unpaired Student's *t* test as a post hoc test), or the Pearson correlation test, as appropriate (24). A two-tailed *P* value < 0.05 was considered significant. All statistical analyses were performed with SPSS version 9.0 (SPSS Inc, Chicago).

### RESULTS

As shown in **Table 2**, there were no significant differences in sex or age between the patients with COPD and the control subjects. PaO<sub>2</sub>, FEV<sub>1</sub>, and the diffusion capacity for carbon monoxide were significantly lower in the patients than in the control subjects, whereas the residual volume and the intrathoracic gas



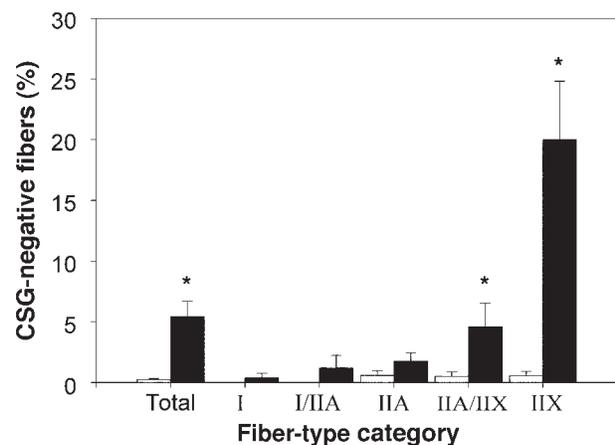
**FIGURE 2.** Overall and type-specific mean ( $\pm$ SE) muscle fiber cross-sectional area in specimens from control subjects (white bars) and patients with chronic obstructive pulmonary disease (black bars). \*Significantly different from control: \* $P < 0.05$ ; \*\* $P < 0.01$ .



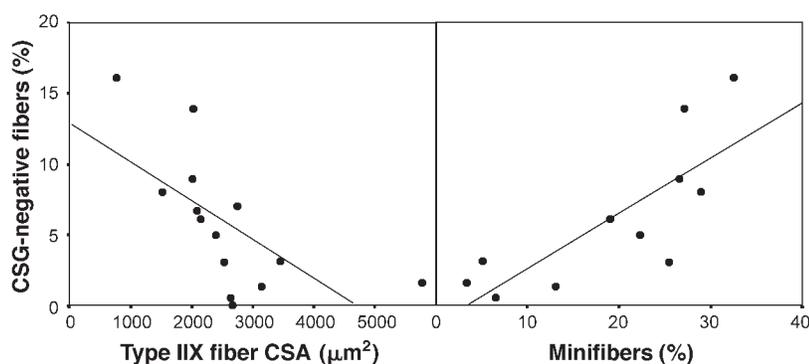
**FIGURE 3.** Relation between muscle fiber cross-sectional area (CSA) and fat-free mass in chronic obstructive pulmonary disease.  $r = 0.87$ ,  $P < 0.001$ .

volume were significantly higher in the patients than in the control subjects. Both the FFM index determined by DXA and body mass index were significantly lower in the patients with COPD than in the control subjects.

A typical example of histochemical results is shown in Figure 1. The mean fiber CSA of 12 control subjects and 14 patients are shown in **Figure 2** (for technical reasons the sections of 4 subjects were not suitable for morphometric analysis). The overall mean fiber CSA in the patients was lower than that in the healthy control subjects (3839 compared with 4647  $\mu\text{m}^2$ ,  $P = 0.037$ ). Within the fiber-type categories, the mean CSA in the IIA/IIX and IIX fiber-type categories was lower in the patients than in the control subjects [3358 compared with 4428  $\mu\text{m}^2$  ( $P = 0.022$ ) and 2566 compared with 4248  $\mu\text{m}^2$  ( $P = 0.003$ ), respectively], whereas there were no differences between the 2 groups in the CSA of type I, I/IIA, and IIA fibers. In addition, there was a higher occurrence of MFs in the patients than in the control subjects (19.1% compared with 7.7%,  $P = 0.005$ ). Atrophy predominantly of type IIA/IIX and IIX fibers is clearly shown by the small dark fibers in Figure 1C.



**FIGURE 4.** Overall and type-specific mean ( $\pm$ SE) proportions of CSG (cytochrome oxidase, succinate dehydrogenase, and glycogen phosphorylase)-negative fibers in specimens from control subjects (white bars) and patients with chronic obstructive pulmonary disease (black bars). \*Significantly different from control,  $P \leq 0.001$ .



**FIGURE 5.** Relation between the proportion of CSG (cytochrome oxidase, succinate dehydrogenase, and glycogen phosphorylase)-negative fibers and type IIX fiber cross-sectional area (CSA) ( $r = 0.65$ ,  $P = 0.012$ ) or the proportion of minifibers ( $r = 0.79$ ,  $P = 0.004$ ).

The body mass index of patients correlated significantly with the total mean fiber CSA ( $r = 0.54$ ,  $P = 0.047$ ). This relation was not found for the control subjects. In addition, total mean fiber CSA correlated even more strongly with the  $FFM_{DXA}$  ( $r = 0.80$ ,  $P = 0.003$ ), the  $FFM_{BIA}$  (Figure 3), and the FFM index determined by BIA ( $r = 0.72$ ,  $P = 0.003$ ) in the patients with COPD. In the control subjects, the  $FFM_{DXA}$  also correlated with the total mean fiber CSA ( $r = 0.58$ ,  $P = 0.048$ ).

Staining for the enzyme activities of COX, SDH, and GlyP remarkably showed the existence of fibers that stained negative (Figure 4). Whereas 5.4% of all fibers were CSG-negative in the patients with COPD, only 0.2% were CSG-negative in the control subjects ( $P = 0.001$ ). CSG-negative fibers were frequently observed in the IIX fiber-type category. In the patients, 20.0% of the type IIX fibers were CSG-negative, whereas in the control subjects, only 0.6% were ( $P = 0.001$ ). A similar result was also found for type IIA/IIX fibers (4.6% in the patients compared with 0.5% in the control subjects,  $P = 0.001$ ). The fibers indicated by arrows in Figure 1, G–I, are examples of CSG-negative fibers.

In the patients with COPD, the proportion of CSG-negative fibers was negatively correlated with the total mean fiber CSA ( $r = -0.60$ ,  $P = 0.024$ ) and with the mean CSA of IIX fibers (Figure 5). A high correlation was also found between the proportions of MFs and CSG-negative fibers in the patients. Note that the CSG-negative fibers in Figure 1 are indeed very small and are identified as type IIX fibers. These correlations were not found in the control subjects.

There were no differences between the patients who were receiving corticosteroids at the time of the study ( $n = 7$ ) and those who were not ( $n = 7$ ) in overall fiber CSA (3703 compared with 3976  $\mu\text{m}^2$ ,  $P = 0.620$ ) or type IIX fiber CSA (2339 compared with 2792  $\mu\text{m}^2$ ,  $P = 0.620$ ). Accordingly, there was no difference between patients with a history of corticosteroid use ( $n = 10$ ) and those without ( $n = 4$ ) in overall fiber CSA (3615 compared with 3929  $\mu\text{m}^2$ ,  $P = 0.607$ ) or type IIX fiber CSA (2306 compared with 2670  $\text{mm}^2$ ,  $P = 0.620$ ). Within the subgroup of patients receiving corticosteroids, there was no correlation between fiber CSA (overall or fiber type-specific) and corticosteroid dose. There were no differences in fiber-type composition or proportions of MFs or CSG-negative fibers between those who had a history of corticosteroid use and those who did not; nor could any correlations between corticosteroid dose and these variables be found.

## DISCUSSION

In this study we clearly show that fiber atrophy significantly contributes to the loss of total body weight and muscle mass in COPD, which is most obvious from the strong correlation between mean fiber CSA and FFM. We also show that fiber atrophy is mainly confined to type IIA/IIX and IIX fibers. Furthermore, a large proportion of the affected fibers lack stainable activity of the oxidative enzymes COX and SDH and the glycolytic enzyme GlyP.

Our finding that atrophy occurs predominantly in type IIA/IIX and IIX fibers is in contrast with data from Whittom et al (4), who found the largest reduction in CSA in type I fibers. However, they reported a normal fiber type I CSA in patients with COPD but a relatively high fiber type I CSA (nearly 7000  $\mu\text{m}^2$ ) in their healthy, age-matched control subjects compared with that in healthy 65-y-old subjects (25–27). Therefore, the observed decrease in fiber type I CSA was probably biased by the aphysiologically high CSA in the muscles of the control subjects. The CSA of type I fibers in both healthy subjects and patients with COPD in our study was 4600  $\mu\text{m}^2$ , which is in the normal range (25). Atrophy predominantly of type II fibers was also found in emphysema patients with severe emaciation compared with control patients or healthy subjects, but this was established by measuring fiber diameter instead of the CSA (6). A disadvantage of this approach is that it is based on the assumption that the fiber cross sections are perfectly round, which is certainly not the case for atrophied fibers, which are often angulated. Moreover, none of these previous studies reported a relation between FFM and muscle fiber CSA.

Hampered by their disease, patients with COPD perform less physical activity, and therefore, disuse may be one of the factors leading to atrophy. On limb unloading or immobilization, overall muscle mass declines, and this decline is initially associated with fiber type I but later with other fiber types as well (8, 28, 29). The influence of unloading on muscle morphology in humans was studied in subjects undergoing a 6-wk unilateral lower limb suspension, which resulted in fiber types I and IIA atrophy (30). In addition, significant fiber type I atrophy and a tendency for fiber type II atrophy were observed in subjects after 6 wk of bed rest (31). These collective findings indicate that disuse cannot—at least not alone—explain the atrophy predominantly of fiber type IIX found in patients with COPD in the present study because disuse causes either atrophy predominantly of fiber type I or generalized muscle fiber atrophy.

In both animals and humans, muscle fiber atrophy occurs in a state of energy deficiency (7). Atrophy predominantly of fiber type II was found in anorexia nervosa (32–34). Animal food-deprivation studies also show muscle fiber atrophy that is more pronounced in type II fibers (35–38). The relative preservation of type I fiber size would be advantageous because there is evidence that the energy expenditure per unit tension developed is lowest in slow-twitch fibers (7). Nutritional depletion is very common in COPD (39–41) and thus, having a negative energy balance could very well contribute to the selective atrophy of type II fibers in patients suffering from this disease. For other chronic diseases, similar results were found with respect to muscle fiber sizes. A mean muscle fiber CSA similar to that found in the present study was reported in patients with chronic heart failure (CHF) (42). In addition, atrophy predominantly of fiber type II was also found in CHF (43, 44), AIDS (45), and chronic renal failure (46–48). As in COPD, malnutrition is a frequent problem in CHF (49, 50), AIDS (51), and chronic renal failure (52), and it is therefore very likely that the selective type II fiber atrophy is caused by a common mechanism.

In the present study there were no differences (not even a tendency) in mean muscle fiber CSA (overall or fiber type-specific) or in the proportion of CSG-negative fibers between the patients who had a history of corticosteroid use and the patients who were receiving corticosteroids at the time of the muscle biopsy; there were also no differences between these 2 groups and the patients who had no history of corticosteroid use. Accordingly, in the subgroup of patients receiving corticosteroids there was no relation between dosage and muscle fiber CSA. The patients in the present study did not receive high doses of oral corticosteroids. We previously found no differences in enzyme activities in the tibialis anterior between patients with COPD who received a low dose of oral corticosteroids and control patients who did not (53). In addition, thigh muscle CSA did not differ between patients who received systemic corticosteroids and those who did not (1). In the past, oral corticosteroids have indeed been associated with skeletal muscle fiber atrophy, and, as in malnutrition, type II fibers are more susceptible than are type I fibers (9, 54). However, animal studies showed that type IIX fiber atrophy is induced by fluorinated corticosteroids (like dexamethasone and triamcinolone) and not by nonfluorinated corticosteroids (55–57). Prednisolone is a nonfluorinated corticosteroid and therefore is unlikely to have caused the marked type IIX fiber atrophy in the present study. Selective fiber type IIX atrophy was shown in the vastus lateralis in 8 patients with COPD who were diagnosed with steroid-induced myopathy (5), but there was probably a selection bias in this study because the patients were selected on the basis of a diagnosis of steroid-induced myopathy (5). Therefore, our data combined with data from the literature suggest that oral corticosteroid treatment in which prednisolone is given at a maintenance dose is not an important factor contributing to muscle fiber atrophy in COPD.

We can only speculate on the molecular mechanisms involved in muscle fiber atrophy in COPD. The fact that many of the atrophied IIX fibers in the present study lacked stainable enzyme activity for COX, SDH, or GlyP suggests that at a certain stage of fiber atrophy muscle cells lose their potential to metabolize the substrates required for ATP synthesis. This inevitably leads to dysfunction of the affected muscle fiber in addition to the loss of functional muscle mass. As in CHF, muscle fiber atrophy may be associated with apoptosis (42), and it has been suggested that

apoptosis is involved in muscle remodeling (58). The loss of muscle mass may also be caused by a reduction in the total number of fibers. We cannot exclude the involvement of such fiber loss; moreover, atrophy may even be a process that precedes and eventually leads to fiber loss. The fact that minifibers lose the activity of enzymes involved in energy metabolism and are probably dying favors this theory.

In summary, we previously showed that FFM is a strong predictor of skeletal muscle weakness in COPD (3). In the present study we show that loss of FFM is associated with muscle fiber atrophy. It can therefore be concluded that muscle fiber atrophy, especially of type IIX fibers, is involved in the loss of muscle mass in COPD and most likely results in muscle weakness. An abnormal metabolic profile is associated with fiber atrophy, but additional research is necessary to further clarify the molecular pathogenesis of muscle wasting in COPD. 

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