Corticosteroid effects on isotonic contractile properties of rat diaphragm muscle

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Van Balkom, Roland H. H., Wen-Zhi Zhan, Y. S. Prakash, P. N. Richard Dekhuijzen, and Gary C. Sieck. Corticosteroid effects on isotonic contractile properties of rat diaphragm muscle. *J. Appl. Physiol.* 83(4): 1062–1067, 1997.—The effects of corticosteroids (CS) on diaphragm muscle (Dia_m) fiber morphology and contractile properties were evaluated in three groups of rats: controls (Ctl), surgical sham and weight-matched controls (Sham), and CS-treated (6 mg·kg^{-1}·day^{-1} prednisolone at 2.5 ml/h for 3 wk). In the CS-treated Dia_m, there was a selective atrophy of type IIx and IIb fibers, compared with a generalized atrophy of all fibers in the Sham group. Maximum isometric force was reduced by 20% in the CS group compared with both Ctl and Sham. Maximum shortening velocity in the CS Dia_m was slowed by ~20% compared with Ctl and Sham. Peak power output of the CS Dia_m was only 60% of Ctl and 70% of Sham. Endurance to repeated isotonic contractions improved in the CS-treated Dia_m compared with Ctl. We conclude that the atrophy of type IIx and IIb fibers in the Dia_m can only partially account for the CS-induced changes in isotonic contractile properties. Other factors such as reduced myofibrillar density or altered cross-bridge cycling kinetics are also likely to contribute to the effects of CS treatment.

prednisolone; skeletal muscle; fiber type; shortening velocity; fatigue; endurance

CORTICOSTEROID (CS) treatment is common in the clinical setting, despite a variety of contraindications, including skeletal muscle myopathy. Recently, considerable attention has focused on the possibility that CS treatment impairs diaphragm muscle (Dia_m) function in patients with chronic obstructive pulmonary disease (1). In these patients, CS treatment appears to contribute to Dia_m weakness, further reducing their functional reserve capacity. To date, animal studies have examined only the effects of CS treatment on isometric properties of the Dia_m. However, an examination of only the isometric properties of the Dia_m may not reveal the true impact of CS treatment. The force-velocity relationship is an essential characteristic of Dia_m contractile properties, and, to date, there is very little information concerning the effects of CS treatment on the ability of the Dia_m to shorten. This may explain the equivocal results of animal studies reporting either no effect of CS treatment on maximum isometric specific force (P_o; force normalized for muscle cross-sectional area) of the Dia_m (2, 3, 10, 13, 22) or only a small reduction in specific force (20).

As in other skeletal muscles, the maximum shortening velocity (V_{max}) of Dia_m fibers displays a strong association with myosin heavy chain (MHC) isoform composition (8, 18). In the Dia_m, type IIx and IIb fibers, expressing the MHC_{2X} and MHC_{2B} isoforms, respectively (16, 19), have a faster V_{max} than type I and IIa fibers, expressing the MHC_{slow} and MHC_{2A} isoforms, respectively. An effect of CS treatment on the force-velocity relationship of the Dia_m is suggested by the selective atrophy of type IIx and/or IIb fibers (2, 3, 12, 14, 20, 22). Accordingly, we hypothesize that, in the Dia_m, CS treatment is associated with a slowing of V_{max}.

Fiber type differences in V_{max} also correspond to differences in power output, with type IIx and IIb fibers generating greater power than type I and IIa fibers (18, 19). If CS treatment selectively affects the size of type IIx and IIb fibers, then the power output of the Dia_m should be reduced. The increased power output of type IIx and IIb Dia_m fibers is also associated with greater energetic demands, compared with type I and IIa fibers (18). Thus a reduction in the relative contribution of type IIx and IIb fibers to total Dia_m mass should result in an overall reduction in energy requirements. If muscle fatigue is related to an imbalance between energy supply and energy demand, the effects of CS treatment may be reflected by an improvement in fatigue resistance (rate of force decline) or endurance (duration of sustained power output). Indeed, previous studies have reported an improvement in isometric fatigue resistance of the rat Dia_m after CS treatment (13, 22). However, since energy requirements increase with power output (4, 18), the effects of CS treatment on improving endurance should be even more pronounced during repetitive isotonic shortening.

In the present study, we evaluated the effects of CS treatment on the isotonic contractile and endurance properties of the rat Dia_m. We hypothesized that CS treatment induces a selective atrophy of type IIx and IIb Dia_m fibers and that, as a result, there is a slowing of V_{max}, a decrease in power output, and an improvement in isotonic endurance.

METHODS

Male Sprague-Dawley rats (initial body weights 315 ± 5 g) were divided into three groups: *1*) untreated controls (Ctl; n = 8); *2*) surgical sham and weight-matched controls (Sham; n = 8); and *3*) CS-treated (CS; n = 8). All animals were housed in separate cages under a 12:12-h light-dark cycle, fed with Purina rat chow, and provided with water ad libitum. Animals in the Ctl and CS groups were provided food ad libitum, whereas rats in the Sham group were food restricted to match their weight growth curve with that of the CS group. Body weights were monitored daily in all groups.

All procedures used in this study were approved by the Institutional Animal Care and Use Committee of the Mayo

Clinic and were in strict accordance with the American Physiological Society animal care guidelines. Surgical procedures were performed under aseptic conditions. The recovery of animals from surgery was carefully monitored.

CS treatment. Animals were anesthetized by the administration of ketamine (60 mg/kg im) and xylazine (2.5 mg/kg im), and a miniosmotic pump (Alzet 2M4) was implanted sucutaneously in the neck. In the CS group, the miniosmotic pump contained a 37.5 mg/ml aqueous suspension of prednisolone sodium succinate (Upjohn), whereas in the Sham group the pump contained a sterile physiological saline solution. Based on a flow rate of 2.5 μl/h for the osmotic pump, a dose of 6 mg/kg prednisolone was provided continuously for a 3-wk period. Measurements of the remaining amount of solution in the pump at the end of the 3-wk treatment period were used to estimate total drug delivery. At the terminal experiment, blood samples were obtained to measure prednisolone, 3,3′,5-triiodo-L-thyronine (T_3), and thyroxine (T_4).

Fiber type composition and morphology. After the 3-wk treatment period, the rats were anesthetized with pentobarbital sodium (70 mg/kg), and the right Dia_m was rapidly excised. Muscle segments were dissected from the midcostal region, and the resting excised length of the strip was measured by using digital calipers. The muscle strips were then stretched to 1.5 times this excised length [an approximation for optimal fiber length (L_o) (15)], pinned on cork, and rapidly frozen in melting isopentane cooled to its melting point by liquid nitrogen.

Transverse sections of muscle fibers were cut at 6 μm by using a cryostat (Reichert Jung 2000E) kept at −20°C. The muscle sections were then reacted with antibodies to different MHC isoforms: *1)* mouse anti-MHC_{slow} immunoglobulin (Ig) G (Novocastra) for identification of type I fibers by positive immunoreactivity; *2)* mouse anti-MHC_{2A} IgG (7) for identification of type IIa fibers by positive immunoreactivity; *3)* mouse anti-$MHC_{all\text{-}2X}$ IgG (16) for identification of type IIx fibers by negative immunoreactivity; and *4)* mouse anti-MHC_{2B} IgM (16) for identification of type IIb fibers by positive immunoreactivity. After a 2- to 3-h incubation with the primary antibody, the sections were washed in 0.1 M phosphate buffer and incubated further in Cy3-conjugated donkey anti-mouse IgG or IgM.

The fluorescently stained sections were visualized by using an Olympus BH-2 microscope. Images of the stained muscle sections were digitized into a 1,024 × 1,024 array of picture elements (pixels) by using a charge-coupled diode camera attached to a calibrated image-processing system (19). With the use of a ×20 microscope objective, each pixel had a projected area of 0.15 μm^2. The cross-sectional area of individual muscle fibers was determined from the number of pixels within the delineated boundary of the fiber. To determine fiber type proportions, ~500 muscle fibers were sampled from each Dia_m. Cross-sectional areas were measured for at least 25 fibers of each type within a given muscle. The relative contribution of each fiber type to the total area of the muscle segment (an estimate of total mass when L_o was similar) was calculated based on the proportion and average cross-sectional area of each fiber type.

MHC isoform composition. The techniques for determination of MHC isoform composition of the rat Dia_m have been previously described (8, 19). Briefly, myosin was extracted from scissor-minced Dia_m tissue, the extracts were centrifuged, and supernatants were recovered. After overnight storage to allow precipitation of myosin filaments, the solution was centrifuged, and the pellet was dissolved in a sample buffer, boiled, and then stored frozen. Different MHC isoforms were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. The identity of specific MHC bands in silver-stained gels had been previously determined by using immunoblotting techniques (9, 19). The relative composition of the different MHC isoforms was determined by densitometry, normalizing the average density of each band for the total peak densities for all the isoforms combined.

Contractile and endurance properties. Muscle strips (~3 mm wide) were dissected from the midcostal region, with fiber insertions at the costal margin and central tendon left intact. The muscle strip was mounted vertically in a glass tissue chamber containing oxygenated mammalian Ringer solution of the following composition (mM): 135 Na^+, 5 K^+, 2 Ca^{2+}, 1 Mg^{2+}, 121 Cl^-, 25 HCO_3^-, 11 glucose, 0.3 glutamic acid, 0.4 glutamate, and *N,N*-bis(2-hydroxyethyl)-2-aminoethane-sulfonic acid buffer (pH = 7.4). A 0.0008% solution of *d*-tubocurarine chloride was added to prevent neuromuscular transmission. The solution was oxygenated with 95% O_2-5% CO_2 and maintained at 26°C. The origin of the muscle bundle along the costal margin was attached to a metal clamp mounted in series with a micromanipulator at the base of the tissue chamber. The central tendon was glued to a thin, stiff plastic rod that was firmly fixed to the lever arm of a dual-mode length-force servo-control system (Cambridge Technologies, model 300B).

The muscle was stimulated directly by using platinum plate electrodes placed in close apposition on either side of the muscle. Rectangular current pulses (0.5-ms duration) were generated by using a Grass S88 stimulator and amplified by a current amplifier (Mayo Foundation, Section of Engineering). The stimulus intensity producing the maximum twitch force response was determined, and the stimulus intensity was set at ~125% of this value for the remainder of the experiment (~220 mA). Muscle preload was adjusted by using the micromanipulator until L_o for maximal twitch force was achieved.

The Cambridge system was controlled by using custom-built software (LabView), implemented on an IBM 486 personal computer. Length and force were independently controlled, allowing the Cambridge system to operate either in isometric or isotonic modes, respectively. Length and force outputs were digitized by using a data-acquisition board (National Instruments) at a sampling frequency of 1 kHz.

Peak isometric twitch force (P_t) and P_o (600-ms duration train) were measured. The force-velocity relationship of the Dia_m was then determined. While the muscle was maximally stimulated at 75 Hz for 330 ms, afterloads were clamped at values ranging from 3 to 100% of P_o. A shorter stimulus duration was used to accommodate the limited range of lever movement of the Cambridge system during muscle shortening. At least 1 min intervened between each load level. The velocity of shortening at each load clamp was calculated as the change in muscle length (normalized for L_o) during a 50-ms period. To eliminate the dynamics of connective and other noncontractile tissue in the muscle, the time window for this measurement was set to begin at 25 ms after the first detectable change in length. V_{max} was calculated by fitting the force-velocity curve by using the modified Hill equation and extrapolating the fitted curve to zero-load (21).

Power output during isotonic contraction was calculated as the product of force and velocity, and the load clamp level yielding maximum power was determined. The load clamp was set to this value, and endurance was assessed during repetitive isotonic shortening induced by stimulating the muscle at 75 Hz in 330-ms duration trains repeated every second. The time at which power output declined to zero (no detectable muscle shortening) was defined as endurance time.

After the experiment, the muscle was weighed, and cross-sectional area was estimated based on the following formula:

muscle weight (g)/[L_o (cm)·1.056 (g/cm^3)]. Forces were then normalized for cross-sectional area of the muscle segments.

Statistical analysis. Data were compared by using a one-way analysis of variance followed by Duncan's multiple-range test. Repeated-measures analysis of variance was used for analysis of force-frequency, force-velocity, and force-power relationships, as well as for the analysis of the decline in maximum power output during the isotonic fatigue test. Statistical significance was tested at the 0.05 level. All data were expressed as means ± SE.

RESULTS

Efficacy of CS treatment. After 3 wk of treatment, there was very little residual solution (<5% of total volume) remaining in the miniosmotic pumps. Prednisolone levels measured in blood serum of Ctl and Sham animals were below detectable levels (<0.5 μg/dl). In contrast, the serum prednisolone level measured in the CS-treated animals at the time of the terminal experiment was 4.9 ± 1 μg/dl. Serum T_3 and T_4 levels were not significantly different across the three experimental groups (Ctl: T_3 46 ± 3 ng/dl, T_4 4.0 ± 0.2 μg/dl; Sham: T_3 48 ± 6 ng/dl, T_4 4.2 ± 0.4 μg/dl; and CS: T_3 47 ± 4 ng/dl, T_4 3.9 ± 0.4 μg/dl).

Body weights. Over the 3-wk experimental period, body weights of Ctl animals increased by 26% (315 ± 7 g initial and 397 ± 9 g final body weights). In the CS and Sham animals, body weight gain was significantly reduced compared with Ctl ($P < 0.05$), increasing by only 6 and 4%, respectively (CS: 319 ± 5 g initial and 338 ± 9 g final body weights; Sham: 313 ± 7 g initial and 327 ± 9 g final body weights).

Fiber type composition and morphology. In all three experimental groups, fiber types could be readily classified by immunoreactivity for the different MHC antibodies. The incidence of coexpression of MHC isoforms appeared to be very low (<1%) in all three groups. However, it was not possible to detect coexpression of MHC_{2X} and MHC_{2B} isoforms by immunohistochemistry, and it is likely that such coexpression was more frequent (19). There were no differences across groups in the proportions of different fiber types (Table 1).

In the CS-treated animals, cross-sectional areas of type IIx and IIb Dia_m fibers were significantly smaller than those of type IIx and IIb fibers in Ctl ($P < 0.05$; Table 1). In contrast, cross-sectional areas of type I and IIa fibers in the CS Dia_m were comparable to similar fiber types in Ctl animals. In the Sham Dia_m, there was a generalized atrophy of all fiber types compared with Ctl ($P < 0.05$; Table 1). Type I fibers in the Sham Dia_m were also smaller than type I fibers in the CS Dia_m ($P < 0.05$; Table 1). Cross-sectional areas of type IIa, IIx, and IIb fibers in the CS Dia_m were comparable to similar fiber types in Sham animals.

In the CS Dia_m, the relative contribution of type I fibers to total Dia_m cross-sectional area increased ($P < 0.05$; Table 1). Otherwise, there were no differences across groups in the relative contribution of different fiber types to total Dia_m cross-sectional area. However, the combined contribution of type IIx and IIb fiber areas was ~60% of total Dia_m cross-sectional area in Ctl and Sham animals but only ~48% in the CS group (Table 1).

Table 1. *Effect of CS treatment on fiber type proportions, cross-sectional areas, and relative contributions to total Dia_m cross-sectional area*

Treatment	Type I	Type IIa	Type IIx	Type IIb
Fiber type proportions, %total				
Ctl	36.1 ± 0.5	32.5 ± 0.7	23.5 ± 1.5	6.8 ± 1.6
Sham	37.4 ± 1.5	31.0 ± 1.2	24.1 ± 1.5	7.5 ± 1.4
CS	40.4 ± 2.2	29.9 ± 1.5	24.1 ± 1.9	5.5 ± 1.5
Fiber cross-sectional area, μm²				
Ctl	875 ± 28	821 ± 35	2,666 ± 163	3,388 ± 263
Sham	600 ± 19*	693 ± 18*	1,710 ± 75*	2,685 ± 186*
CS	772 ± 62†	770 ± 67†	1,668 ± 202*	2,284 ± 307*
Relative contribution to total Dia_m area, %total				
Ctl	21.7 ± 0.9	18.6 ± 0.6	44.2 ± 4.1	15.5 ± 3.8
Sham	21.6 ± 1.6	20.6 ± 2.0	39.5 ± 3.3	19.2 ± 4.1
CS	29.8 ± 2.3*†	21.7 ± 1.4	37.2 ± 3.2	11.3 ± 3.1

Values are means ± SE. Ctl, control group; Sham, Sham group; CS, corticosteroid-treated group; Dia_m, diaphragm muscle. *$P < 0.05$ compared with Ctl group. †$P < 0.05$ compared with Sham group.

MHC isoform composition. On the basis of electrophoretic separation, the relative expression of the MHC_{2B} isoform decreased in the CS-treated Dia_m ($P < 0.05$; Table 2). The MHC isoform composition of Ctl and Sham Dia_m was comparable (Table 2).

Contractile and endurance properties. After 3 wk of CS treatment, P_t and P_o of the Dia_m were reduced compared with both Ctl and Sham groups ($P < 0.05$, Table 3). P_t and P_o were not different between Ctl and Sham animals. Compared with Ctl and Sham groups, the force-velocity relationships of the CS Dia_m were shifted to the left ($P < 0.05$; Fig. 1*A*). The V_{max} of the CS Dia_m was significantly slower than that of both Ctl and Sham Dia_m ($P < 0.05$, Fig. 1*B*). In all Dia_m, peak power output occurred at ~33% of P_o and at 33% of V_{max} (Fig. 2). Peak power output of the CS Dia_m was significantly lower than that of both Ctl and Sham groups (Fig. 2; $P < 0.05$). The peak power output of the Sham Dia_m was also slightly lower than that of Ctl animals (Fig. 2; $P < 0.05$).

With repetitive shortening contractions, maximum power output of the Dia_m rapidly declined in all three groups (Fig. 3; $P < 0.05$). After 60 s of repetitive contractions, Dia_m power output was comparable in all three groups (Fig. 3). However, given the differences in the initial peak power output of each group, the rate of decline in power was slower in the CS Dia_m compared with both Sham and Ctl animals (Fig. 3; $P < 0.05$).

Table 2. *Effect of CS treatment on MHC isoform composition of Dia_m (%total MHC)*

Treatment	MHC_{slow}	MHC_{2A}	MHC_{2X}	MHC_{2B}
Ctl	22.5 ± 1.1	29.0 ± 1.2	34.1 ± 1.2	14.5 ± 1.9
Sham	24.0 ± 2.3	30.2 ± 1.8	31.6 ± 2.0	14.2 ± 2.1
CS	25.3 ± 1.9	34.8 ± 1.5	33.4 ± 1.0	6.5 ± 1.5*†

Values are means ± SE. MHC, myosin heavy chain. *$P < 0.05$ compared with Ctl, †$P < 0.05$ compared with Sham group.

Table 3. *Effect of CS treatment on isometric contractile properties of the* Dia_m

Treatment	P_t, N/cm²	P_o, N/cm²	P_t/P_o
Ctl	10.5 ± 0.7	21.1 ± 1.5	0.50 ± 0.02
Sham	11.4 ± 0.7	20.3 ± 1.0	0.56 ± 0.02
CS	7.4 ± 0.6†	16.6 ± 0.9*†	0.44 ± 0.02*†

Values are means ± SE. P_t, peak twitch force; P_o, maximum tetanic force. *$P < 0.05$ compared with Ctl; †$P < 0.05$ compared with Sham group.

Endurance time of the CS Dia_m was 120 ± 6 s compared with 96 ± 4 s for Ctl ($P < 0.05$) and 108 ± 7 s for Sham (Fig. 3).

DISCUSSION

The results of the present study support our hypotheses that CS treatment induces a selective atrophy of type IIx and IIb fibers in the rat Dia_m, which is associated with a slowing of V_{max}, a reduction in power output, and an improvement in isotonic endurance. However, the CS-induced changes in Dia_m isotonic properties were disproportionately greater than the changes in type IIx and IIb fiber morphology and MHC isoform expression. Therefore, we conclude that, in addition to the selective atrophy of type IIx and IIb fibers, CS treatment exerts an influence on cross-bridge cycling kinetics.

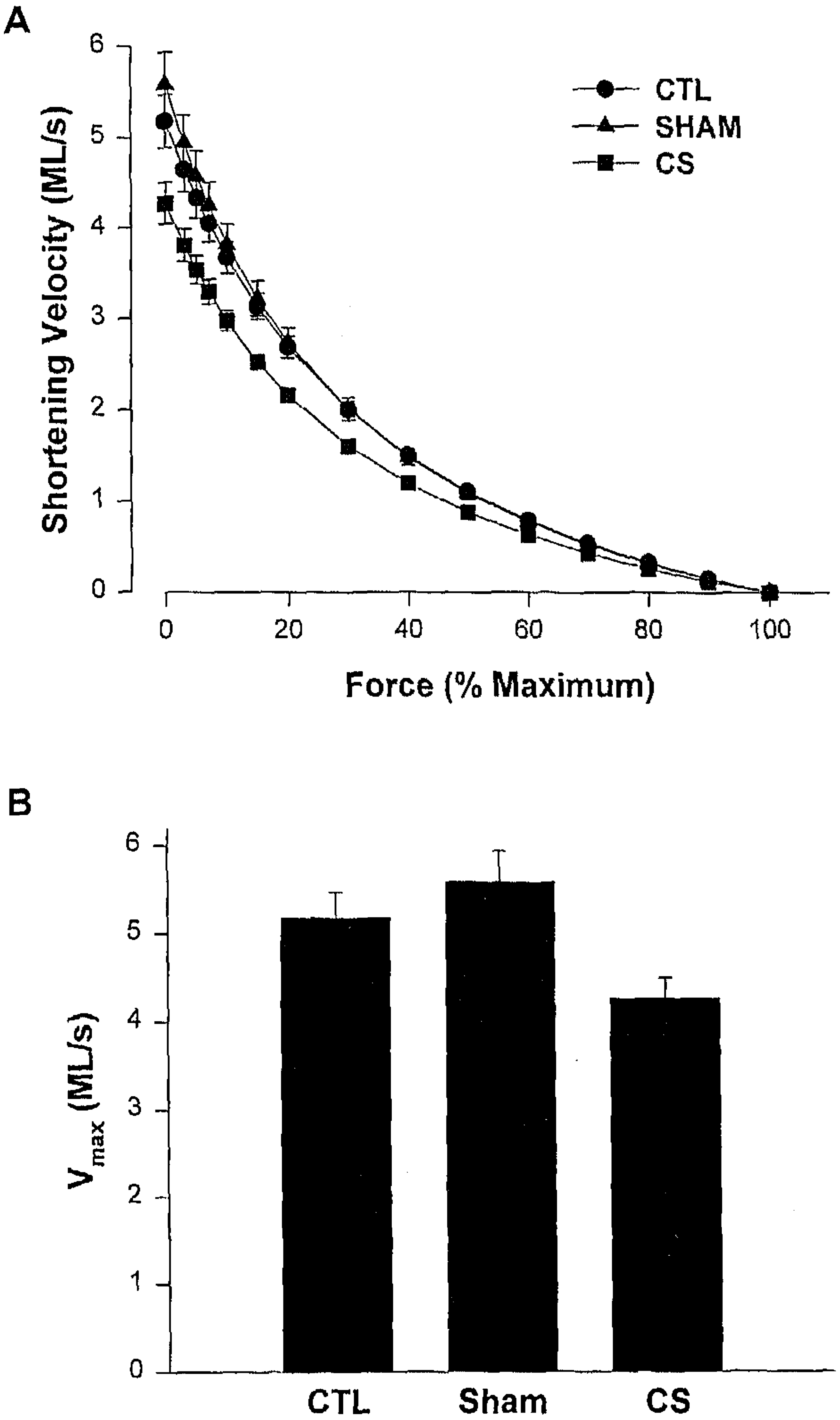

Fig. 1. *A*: force-velocity relationships of corticosteroid-treated (CS) diaphragm muscle (Dia_m) were shifted leftward compared with both control (Ctl) and surgical sham and weight-matched controls (Sham) animals ($P < 0.05$). *B*: maximum shortening velocity (V_{max}) was slower in CS Dia_m compared with both Ctl and Sham groups ($P < 0.05$). ML, muscle lengths.

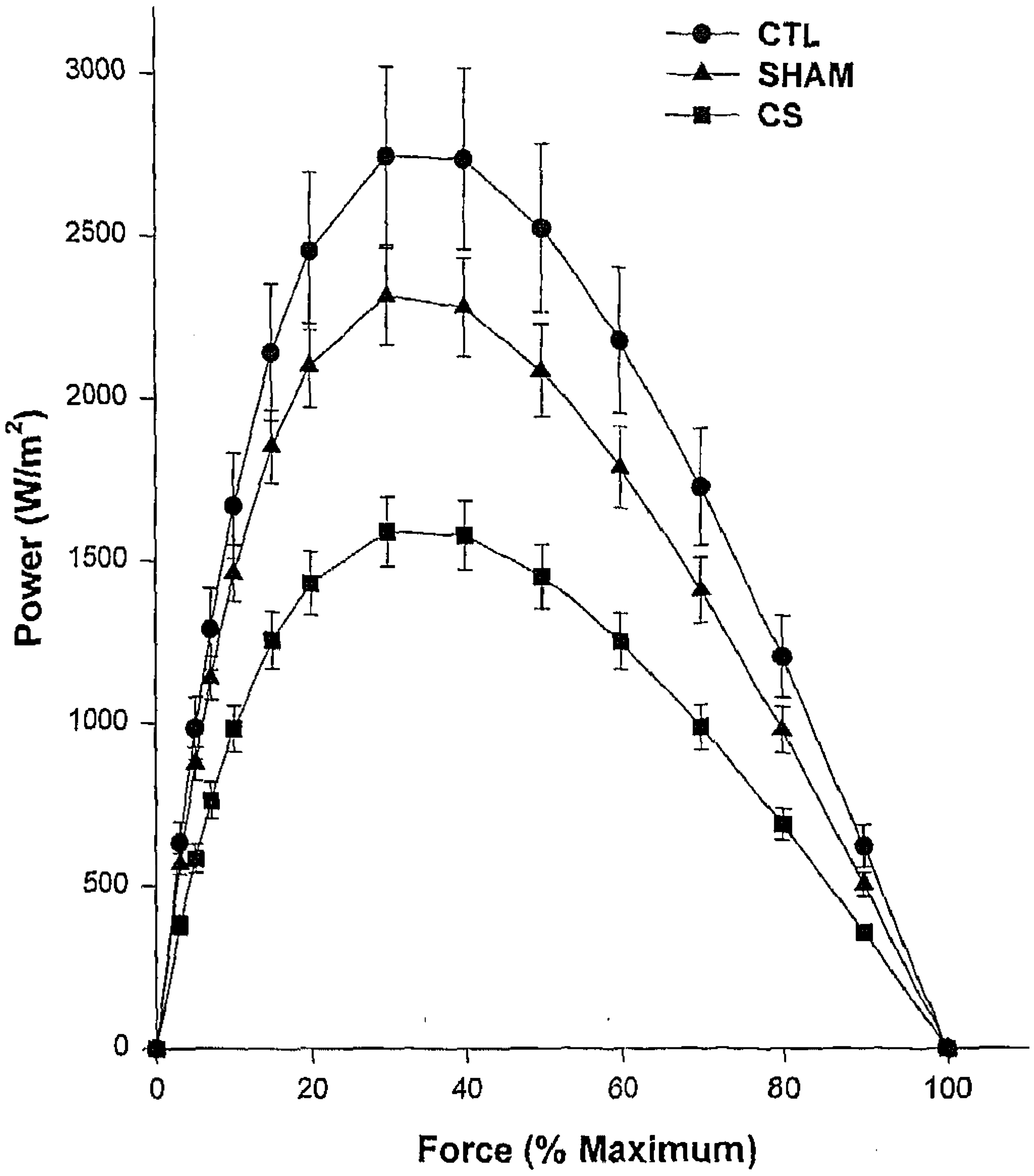

Fig. 2. Power output at each load was reduced in CS and Sham groups compared with Ctl ($P < 0.05$). In addition, power output of CS Dia_m group was less than that of the Sham group ($P < 0.05$). Peak power output occurred at ~33% maximum tetanic force in each group and was reduced in CS and Sham groups compared with Ctl ($P < 0.05$). Peak power output of CS Dia_m group was also lower than that of Sham rats ($P < 0.05$).

Across the 3-wk period, the normal increase in body weight observed in Ctl rats was blunted by CS treatment. The final body weight of the CS-treated animals was ~15% lower than that of Ctl rats. Because alterations in nutritional status alone can affect morphology and function of the rat Dia_m (2, 11, 17), interpretation of the direct effects of CS treatment is confounded. However, the morphological and contractile adaptations of the Dia_m in the Sham group, where body weight was matched to that of the CS group by food restriction, were generally dissimilar to those observed in the CS-treated animals. These results suggest that the effects of CS treatment on Dia_m structural and functional properties cannot be solely attributed to a nonselective catabolic effect.

The CS-induced selective atrophy of type IIx and IIb Dia_m fibers observed in the present study is in general agreement with several previous studies (2, 3, 12, 14, 20, 22). However, these previous studies did not classify fiber types based on expression of different MHC iso-

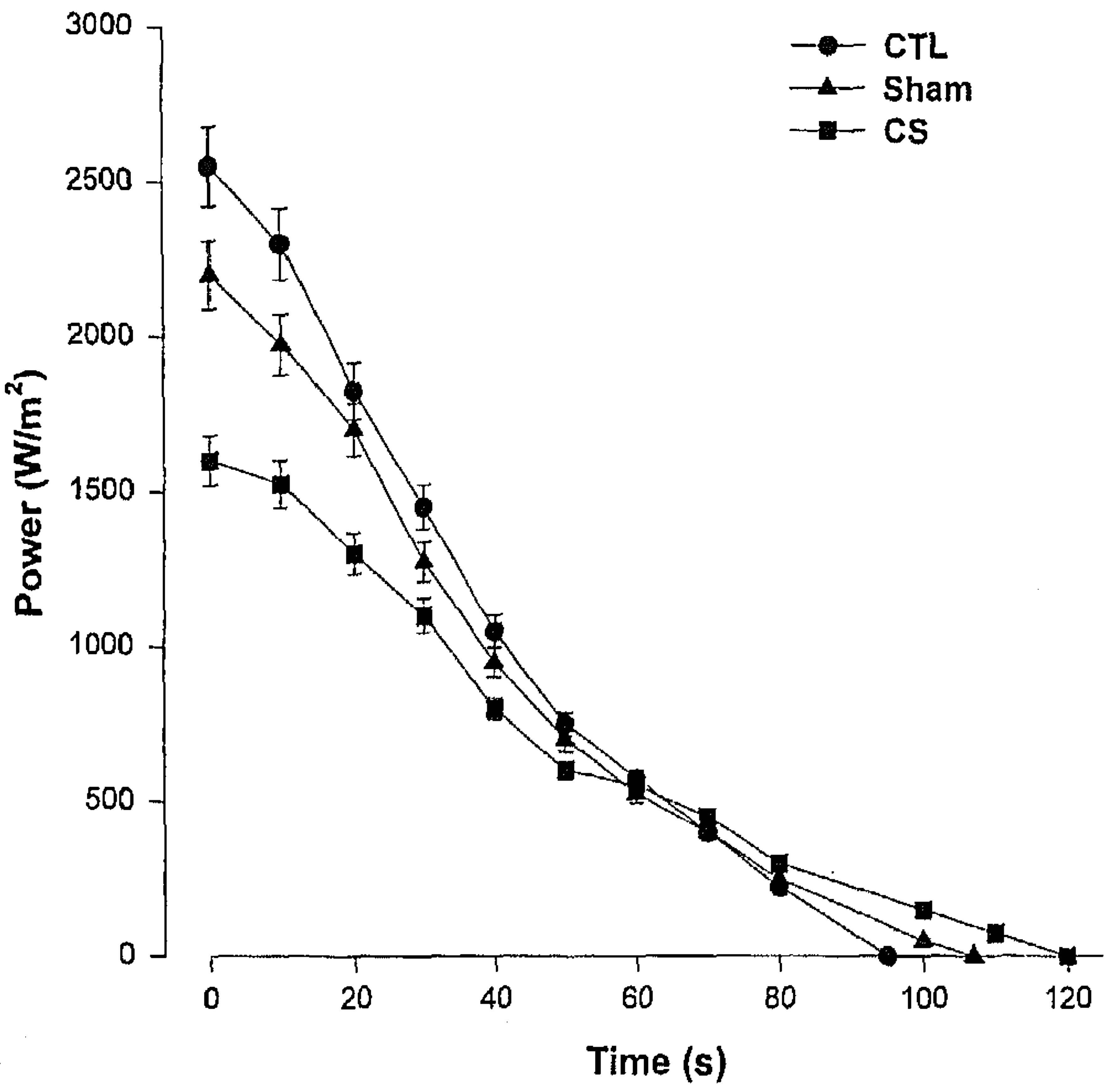

Fig. 3. During repetitive isotonic shortening at 30% maximum tetanic force, power output of the Dia_m rapidly declined in all 3 groups ($P < 0.05$). Rate of decline of power was slower in CS Dia_m compared with Ctl and Sham groups ($P < 0.05$). Endurance time of CS Dia_m group was prolonged compared with Ctl group ($P < 0.05$) but was not significantly different from Sham group.

form, nor did they distinguish between type IIx and IIb fibers. Standard histochemical classification of fiber types based on the pH lability of myofibrillar adenosinetriphosphatase (ATPase) staining, as used in these studies, cannot distinguish type IIx fibers, which are abundant in the rat Dia_m (9, 19). In addition, the MHC_{2B} isoform is often coexpressed with the MHC_{2X} isoform. Therefore, it is not surprising that type IIx and IIb fibers displayed a similar pattern of atrophy in response to CS treatment. In the Sham Dia_m, there was a generalized atrophy of all fiber types, which has also been previously observed (2, 10, 11, 17, 20, 22). When combined, type IIx and IIb fibers comprised ~60% of both Ctl and Sham Dia_m but only ~48% of the CS Dia_m. In the CS-treated Dia_m, there was a reduction in the relative expression of the MHC_{2B} isoform, whereas no changes in MHC isoform expression were observed in the Sham group. When combined, MHC_{2X} and MHC_{2B} isoforms comprised ~40% of the CS Dia_m compared with ~49% of Ctl and ~46% of Sham Dia_m. The relatively modest change in MHC isoform composition of the CS Dia_m was consistent with the normal T_3 and T_4 levels of these animals. Clearly, the CS-induced morphological adaptations of type IIx and IIb Dia_m fibers and the alterations in MHC isoform expression were not as pronounced as the changes in isotonic contractile properties.

Three weeks of CS treatment resulted in a 20% reduction in P_o compared with both Ctl and Sham groups. These results are in agreement with the previous report of van Balkom et al. (20) but contrast with several other studies in which no effect of CS treatment on Dia_m P_o was observed (2, 3, 10, 13, 22). The reasons for these discrepant results are unclear but may relate to the type, dose, and duration of CS treatment used. It is unlikely that the reduction in specific force of the CS-treated Dia_m observed in the present study was attributable only to the selective atrophy of type IIx and IIb fibers or the reduction in MHC_{2B} isoform expression. A reduction in specific force could also arise from a number of alternative mechanisms, including a decrease in myofibrillar density and/or changes in cross-bridge cycling kinetics. Lieu and colleagues (12) reported that CS treatment is associated with a reduction of myofibrillar and sarcoplasmic protein concentration in the rat Dia_m, albeit not as pronounced as in the plantaris muscle. Such alterations in myofibrillar and sarcoplasmic protein concentration could reflect a decrease in the number of available cross bridges and/or changes in calcium handling.

The force-velocity relationship of the Dia_m was altered by CS treatment such that V_{max} was slowed by ~20% and peak power output was reduced by 40% compared with Ctl animals. The slowing of V_{max} in the CS Dia_m is generally consistent with the selective atrophy of type IIx and IIb fibers and the reduction in MHC_{2B} expression. However, the slowing of V_{max} induced by CS treatment was substantially greater than that which would be predicted by the relatively modest reduction in MHC_{2B} expression. Therefore, it is unlikely that the slowing of V_{max} in the CS-treated Dia_m was solely attributable to a selective atrophy of type IIx and IIb fibers and/or the reduction in MHC_{2B} expression. In muscle fibers, V_{max} is correlated with actomyosin ATPase activity (18) and cross-bridge cycling rate. Type I and IIa fibers have lower actomyosin ATPase activities than type IIx and IIb fibers (18, 19) and, as a result, a slower V_{max}. It is possible that the slowing of V_{max} in the CS-treated Dia_m reflects a decrease in actomyosin ATPase activity of muscle fibers independent of MHC isoform expression.

In all groups, the Dia_m displayed very rapid fatigue during repetitive isotonic contractions at a load corresponding to peak power output. During shortening contractions, muscle fiber energy utilization increases (4, 18); thus the rapid fatigue may be related to an imbalance between energy utilization and energy production. CS-treated animals displayed a slower rate of power decrement during repetitive isotonic contractions compared with Ctl and Sham groups and prolonged endurance time compared with Ctl rats. These results are in general agreement with the improved fatigue resistance during repetitive isometric contractions noted in previous studies (13, 22). However, the results of the present study are in contrast to the report of Ferguson and colleagues (5), who found that CS-treated rabbits displayed less endurance to an incremental inspiratory threshold load. However, Dia_m fatigue was not directly verified in this study, and respiratory failure, used to define endurance, could have resulted from a number of mechanisms other than Dia_m fatigue.

The results of the present study suggest that CS treatment reduces energy utilization during repetitive isotonic contractions and thus improves the balance between energy supply and energy demand. A reduction in energy utilization would result from the selective atrophy of type IIx and IIb fibers, which have higher actomyosin ATPase activities (18, 19). In addition, as suggested above, CS treatment may directly reduce actomyosin ATPase activity independent of MHC isoform expression. Other studies have also suggested that CS treatment impairs muscle energy utilization. For example, after CS treatment, there is an accumulation of glycogen (5) and a reduction in creatine kinase activity (6). There may also be an effect of CS treatment on energy production. For example, it has been reported that CS treatment reduces citrate synthase activity in the rat Dia_m (12, 20). However, no effect of CS treatment on succinate dehydrogenase activity was observed (10).

In conclusion, CS treatment causes a reduction in specific force, a slowing of V_{max}, a decrease in power output, and an improvement in endurance during repetitive isotonic contractions. These contractile adaptations are generally consistent with the selective atrophy of type IIx and IIb fibers and the reduction in MHC_{2B} expression that was observed in the CS Dia_m. However, the contractile adaptations are disproportionately greater than the morphological changes induced by CS treatment. Therefore, we conclude that the impairment of Dia_m function associated with CS treatment involves additional mechanisms including a reduction in myofibrillar density and/or a slowing of cross-bridge cycling kinetics.

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