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Original Articles

Myoglobin Levels in Individual Human Skeletal Muscle Fibers of Different Types[1]

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An attempt was made to determine the relationship of myoglobin content to specific fiber types in human muscle. Biopsies were obtained from biceps brachii, vastus lateralis, and gastrocnemius muscles of untrained subjects and from the vastus lateralis muscle of a highly trained athlete at peak training and at intervals of no training (detraining). Individual muscle fibers were assayed, by quantitative microanalytical methods, for myoglobin, lactate dehydrogenase, malate dehydrogenase, citrate synthase, β-hydroxyacyl-coenzyme A dehydrogenase, and adenylokinase activities all on the same fiber. The enzyme levels were used to classify the fibers into type I or II. The results show that the content of myoglobin in human muscle does not differ greatly between fiber types in contrast to other species. The type II fibers contained, on the average, at least two-thirds as much myoglobin as type I fibers. The concentration of myoglobin did not change in either fiber type during detraining (84 days), despite marked changes in lactate dehydrogenase, adenylokinase and the three oxidative enzymes.

KEY WORDS: Myoglobin; Fiber types; Human muscle; Quantitative microchemistry.

Introduction

From the earliest accounts of biochemical diversities among muscles, myoglobin content and muscle color has been considered characteristic of functional type (18). Most present day classification systems correlate myoglobin with oxidative enzyme levels (2), as indeed, myoglobin serves oxidative-phosphorylation by storing oxygen and transporting it to the mitochondria (26), and has been shown to be directly related to oxygen consumption in exercising muscle (4). In many animals, including rat (9), guinea pig (22), chickens, rabbits, and cats (7), myoglobin levels are high in muscles of predominately type I or IIA (highly oxidative) fibers and very low in those of predominately IIB fibers (glycolytic). It is unclear whether this relationship exists in human muscle fiber types. The content of myoglobin in human muscle fibers has been estimated with histochemical techniques, however, the results do not agree. A report of myoglobin localization using benzidine–peroxidase indicated much higher levels in type I than type II fibers (7), whereas a fluorescent antibody method revealed no differences (17). Quantitative analysis can be made in whole muscle homogenates of species, such as rat, guinea pig, and chickens, having certain muscles in which one fiber type predominates (1). However, all human muscles are mixtures of fiber types (16). Jansson and Sylvén (14) quantitatively measured myoglobin in pools of type I and II human fibers and found a modest difference between the groups. The study did not discern, however, whether the type II group was a mixture of fibers with high and low myoglobin levels. A solution would be to analyze individual fibers of known types. This article describes a radioimmunoassay sensitive enough for this purpose and provides data on the myoglobin content in type I and II fibers of 6 muscle biopsies from normal humans in various states of exercise conditioning.

Methods

Participants. Samples of vastus lateralis, biceps brachii, and gastrocnemius muscles were obtained under local anesthesia by the needle biopsy technique from two normal untrained male volunteers, age 30 and 24. Additionally, biopsies of vastus lateris muscle were obtained from one highly trained male cyclist, age 47, at peak training and two additional times during prolonged restriction of physical activity (detraining). The training consisted of 24 months of an uninterrupted regimen incorporating continuous and high intensity intermittant exercise. The regimen was designed to recruit all muscle fiber types and was described in detail previously (2). Maximal oxygen uptake at peak training was 4.1 liters/min (54 ml/kg/min) and after detraining 3.7 liters/min (45 ml/kg/min). Detraining involved restriction of physical exertion to slow walking and was earnestly implemented for 84 days. Written informed consent explaining the purpose of the investigation and the experimental procedures was obtained from all participants.

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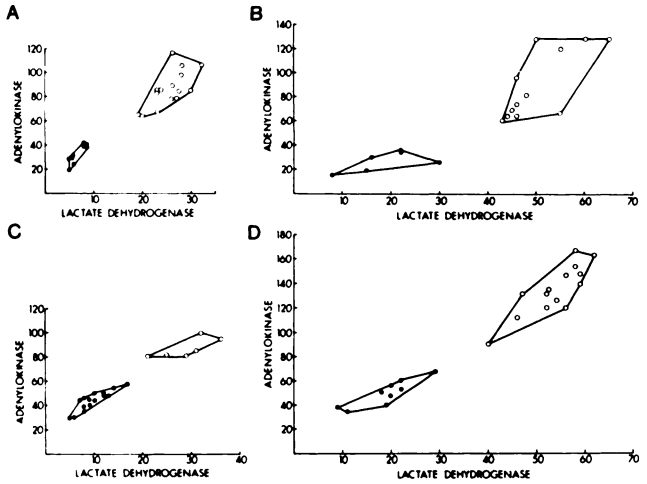


Figure 1. Activities of two enzymes (mol/kg dry wt/hr) used for the purpose of fiber typing, in the same individual human muscle fibers: (A) Vastus lateralis muscle of a normal male; (B) biceps brachii of a normal male; (C) Vastus lateralis of a highly trained male athlete; (D) the same muscle of the same athlete as C after 84 days of detraining. Solid symbols are type I fibers; open symbols are type II.

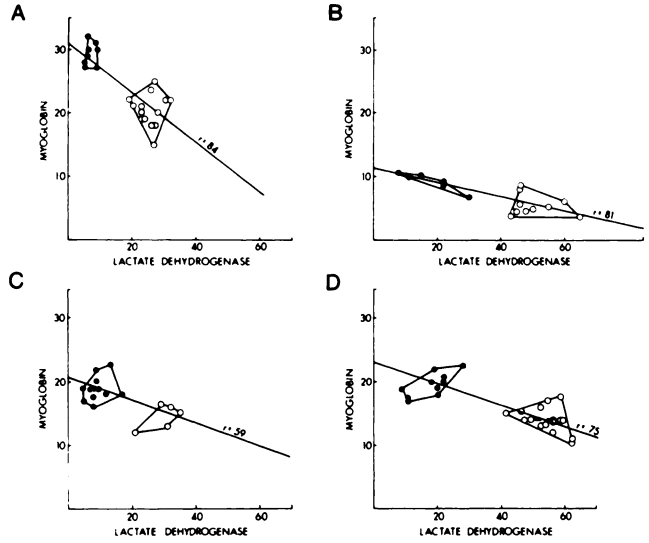


Figure 2. Lactate dehydrogenase activity (mol/kg dry wt/hr) and myoglobin content (mg/g dry wt) in the same individual fibers as in Figure 1.

Enzyme assays. Muscle samples were frozen immediately after biopsy in liquid N_2 cooled to near its freezing point. The samples were freeze-dried at −35°C under vacuum for 7 days and stored under vacuum at −70°C, at treatment that has been shown to prevent loss of enzyme activities (19). For analysis, segments of individual fibers were dissected from the muscle bundles and freed from fat and connective tissue (6). Fiber pieces weighing 40–60 ng were cut off by hand and weighed on a quartz fiber fishpole balance. The method for measuring malate dehydrogenase (MDH) is from Hintz et al. (12) and the methods for measuring lactate dehydrogenase (LDH), adenylokinase (AK), citrate synthase (CS), and β-hydroxyacyl-coenzyme A (CoA) dehydrogenase (BOAC) are from Lowry et al. (19). Further details of the microanalytic procedures are given by Lowry and Passoneau (20).

Myoglobin determination. A microanalytical assay for measuring human myoglobin in single muscle fibers was developed from a commercial radioimmunoassay kit for serum myoglobin (Nuclear Medical Systems, No. 1025). The basic radioimmunoassay has been described by Rosano and Kenney (23) and Stone et al. (24). The following procedure was applied, which includes modifications of the commercial kit to achieve the required sensitivity for the small samples in this study.

Weighed samples were inserted with a fine hair point into the bottom of 7 × 70 mm glass test tubes. A 3 μl aliquot of medium containing 50 mM imidazole–HCl, pH 7.0, 0.05% bovine serum albumin (BSA), and 0.6 M KCl was pipetted onto each sample, as well as into extra tubes for the standards, and incubated for 30 min at 20–25°C to dissolve the myoglobin. This preincubation was necessary to obtain consistent myoglobin values for different sample sizes of the same fibers (without preincubation, myoglobin concentrations were lower in large samples of the fiber than in small samples). Next, the solutions and standards prepared by Nuclear Medical Systems were used at room temperature in the following sequence with the recommended volumes sharply reduced. Ten microliters of the buffer solution was added to each sample. Ten microliters of standard solutions containing 0.156, 0.312, 0.625, and 1.25 ng of myoglobin was added to separate tubes. In addition, a nonspecific binding tube without myoglobin was included. Anti-myoglobin serum from rabbit was added to each tube except the blank in a volume of 10 μl and the Ab-Ag reaction was allowed to proceed for 30 min. Ten microliters of a solution of ^{125}I-myoglobin (approximately 26 ng), was then added and incubated for 1 hr. A 20 μl aliquot of the second antibody, sheep anti-rabbit serum, was added to precipitate the antibody-bound myoglobin. After 30 min, the assay mixture was diluted with 500 μl of distilled water. The unbound ^{125}I-myoglobin was then removed by aspiration of the supernatant immediately after centrifugation for 5 min at 16,000 rpm (Clay-Adams Micro-hematocrit centrifuge). For the precise aspiration we used a fine tipped pipette attached to a vacuum line. Finally, the bound ^{125}I-myoglobin in the precipitated

Table 1. *Myoglobin levels in Type I and Type II human muscle fibers*[a]

	Muscle				
Fiber type	A	B	C	D	E
I	29.25 ± .65	9.20 ± .59	19.02 ± .56	19.89 ± .65	9.76 ± 1.08
II	20.40 ± .65	5.52 ± .50	14.52 ± .86	13.43 ± .51	8.28 ± 1.02
I/II	1.43	1.67	1.31	1.48	1.18

[a]The fibers in muscles A–D are the same as those shown in the figures; E is an additional muscle, the gastrocnemius of a normal male, not completely studied with regard to the oxidative enzymes. Myoglobin levels are mg/g dry wt ± SE.

pellet was counted 5 min on a gamma counter (Beckman, Gamma 300 System). The amount of myoglobin was calculated from a standard curve obtained with each assay. Four determinations were made for each fiber and each standard.

Incubation times for each step were tested for maximal reactivity. The final volume of water was large compared to the precipitate in order to dilute the unbound ^{125}I-myoglobin and provide a more complete removal. In the final step, it is essential to pack the precipitate with high force and to aspirate the supernatant quickly and carefully because the bound ^{125}I-myoglobin pellet tends to loosen on standing and is easily disrupted or dispersed.

Cross-reactivity of the myoglobin antibody was reported by the manufacturer of the kit to be less than 0.1% with human hemoglobin A, citrate synthase, creatine phosphokinase, lactate dehydrogenase, glucose-6-phosphate dehydrogenase, and cytochrome c. Further specificity for human myoglobin was confirmed by our test showing no reaction with 50 ng samples of type I rat muscle fibers.

Myoglobin was measured on pieces of the same muscle fibers after exposure to room temperature for 1 week and to −70°C for 2 months without any significant difference in results. A test was made on 2 fibers cut into 8 and 12 pieces to examine uniformity of myoglobin levels along the fiber length. The concentrations were 26.7 ± 1.4 and 21.5 ± 1.4 mg/g dry wt ± SD in the two fibers, giving 5.2 and 6.5% coefficients of variation.

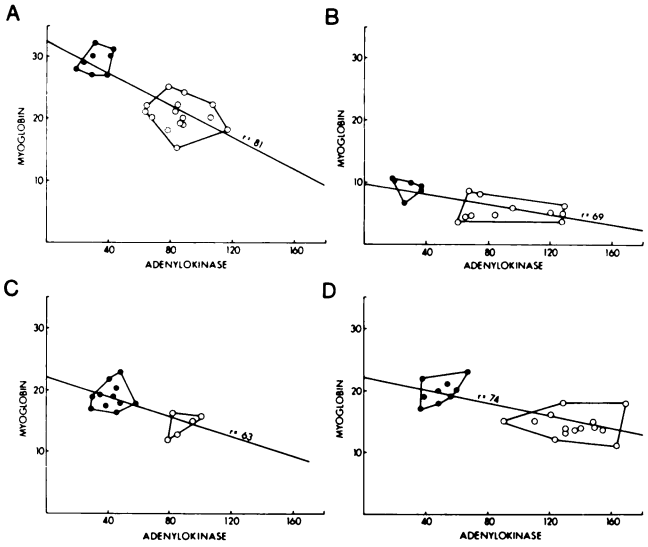


Figure 3. Adenylokinase activity (mol/kg wt/hr) and myoglobin content (mg/g dry wt) in the same fibers as in Figure 1.

Results

The enzymes lactate dehydrogenase and adenylokinase were used to classify individual fibers of the muscle biopsies. Figure 1 gives the enzyme composition of the fiber samples from which myoglobin was subsequently measured. Fibers low in both enzymes are type I, and those high in both are type II. From the random sample, it is clear that the muscles are mixtures of both types, and that a few fibers are intermediate between the two groups.

Our original expectation was that type I fibers would contain much more myoglobin than type II. In fact, although the levels are higher on the average in type I, the differences are not great. The average myoglobin content was only 44% higher in type I than in type II fibers (Table 1), and in one muscle (E) the difference was not statistically significant. Note the wide range in absolute myoglobin levels (see below).

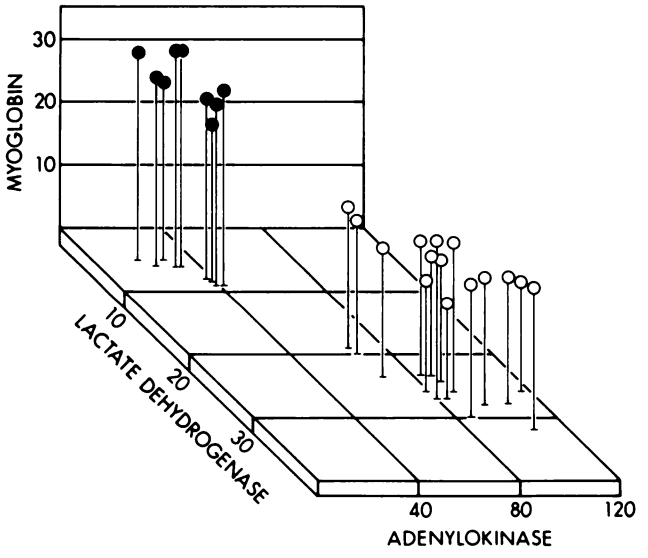
Figures 2 and 3 show the relationship between myoglobin and the two enzymes used for typing the individual fibers. Although the enzyme activities separate the fiber groups completely, the myoglobin levels in the two groups overlap in three of the four cases.

As expected, myoglobin was negatively correlated with enzymes of glycolysis and high energy phosphate metabolism for the fiber population as a whole. However, within the fiber type groups there was no clear or consistent relationship between myoglobin and either enzyme. Muscle A of Figures 2 and 3 is displayed three dimensionally with myoglobin and the two typing enzymes (Figure 4) to further demonstrate the extent of the differences between the fiber type groups and the heterogeneity of enzymes and myoglobin within groups.

Myoglobin content is plotted against oxidative enzymes in individual fibers in Figures 5–7. In most cases, there is a positive and a fairly high correlation between the enzymes and myoglobin level within the fiber population as a whole. However, there is considerable overlap in both myoglobin and oxidative enzymes for type I and type II fiber groups. Thus, neither measure can be used to clearly differentiate human fiber types.

Large individual differences were found in absolute myoglobin levels in the different muscles. This is probably not due to differences in the training state of the muscle, since fibers from the same muscle of one individual (muscles C and D in Figures 2, 3, 5–7) show no significant differences in myoglobin levels under extreme differences in his training state; myo-

Figure 4. Activities of lactate dehydrogenase and adenylokinase (mol/kg dry wt/hr) and the myoglobin content (mg/g dry wt) in the individual fibers of Figure 1A. Type I fibers are clustered at upper left and type II fibers at lower right. The vertical axis (length of each line) gives the level of myoglobin in each fiber.



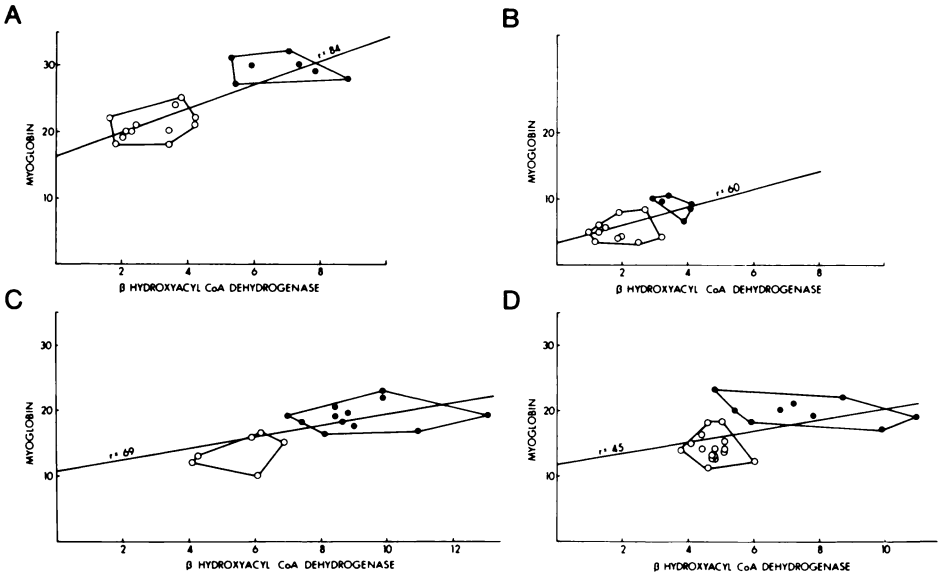


Figure 5. β-Hydroxyacyl-CoA dehydrogenase activity (mol/kg dry wt/hr) and myoglobin content (mg/g dry wt) in the same fibers as in Figure 1.

globin was not changed in either fiber type after 6 or 84 days of detraining (Figure 8, Table 1). This lack of response to detraining in myoglobin is seen despite marked changes in adenylokinase, lactate dehydrogenase, and the three oxidative enzymes on the same fibers (Figure 9). Myoglobin concentration at peak training was unchanged in both types.

Discussion

The enzymes lactate dehydrogenase and adenylokinase can be used to classify individual fibers of human muscle (19). Type I fibers (low in adenylokinase and lactate dehydrogenase) and type II fibers (high in both enzymes) have other energy-related enzyme levels corresponding to the slow-twitch oxidative type I and fast-twitch glycolytic type II fiber types of Peter et al. (22). Moreover, classification on the basis of the above two enzymes agrees well with classification by myosin adenosine triphosphatase (ATPase) staining (9). Quantitative enzymatic typing with these enzymes provides two distinct nonoverlapping groups of human fibers (Figure 1). Although type II skeletal muscle fibers can be subdivided into type IIA (oxidative-glycolytic) and type IIB (glycolytic), the groups are less pronounced in human fibers. This is because oxidative enzyme activities do not differ much between the two major fiber groups (19). The present results show that the same is true for myoglobin.

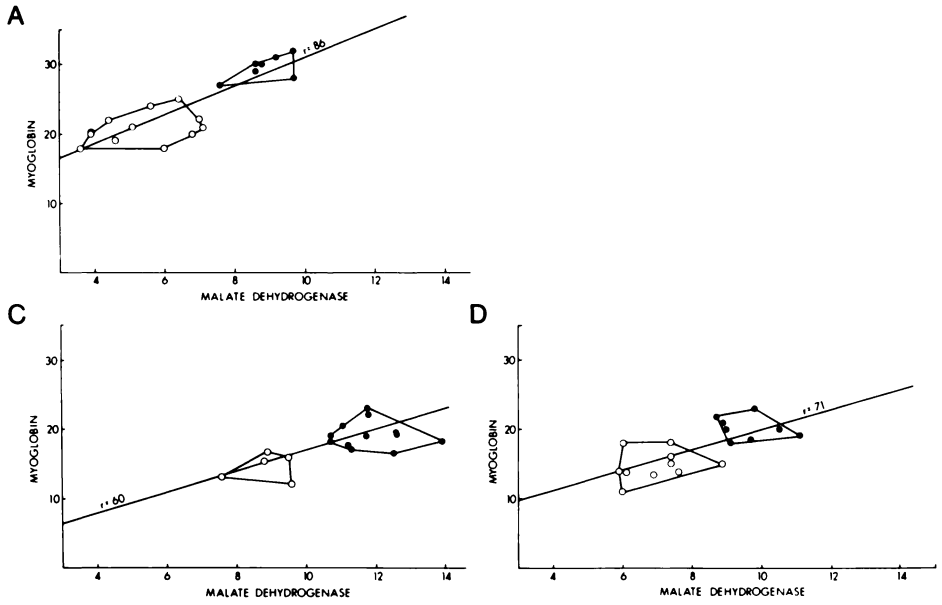


Figure 6. Malate dehydrogenase activity (mol/kg dry wt/hr) and myoglobin content (mg/g dry wt) in the same fibers as in Figure 1.

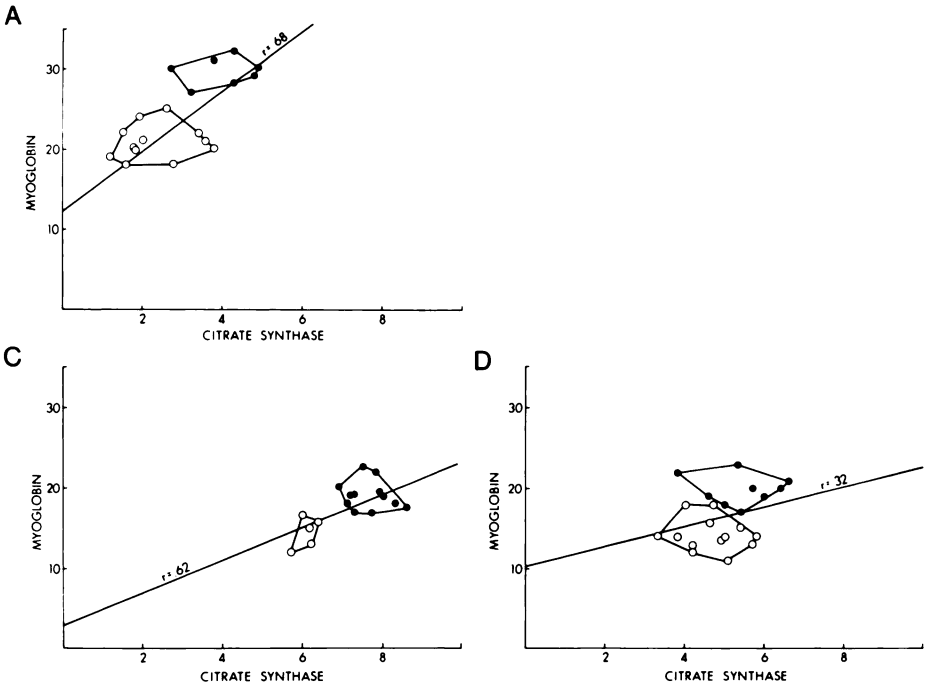


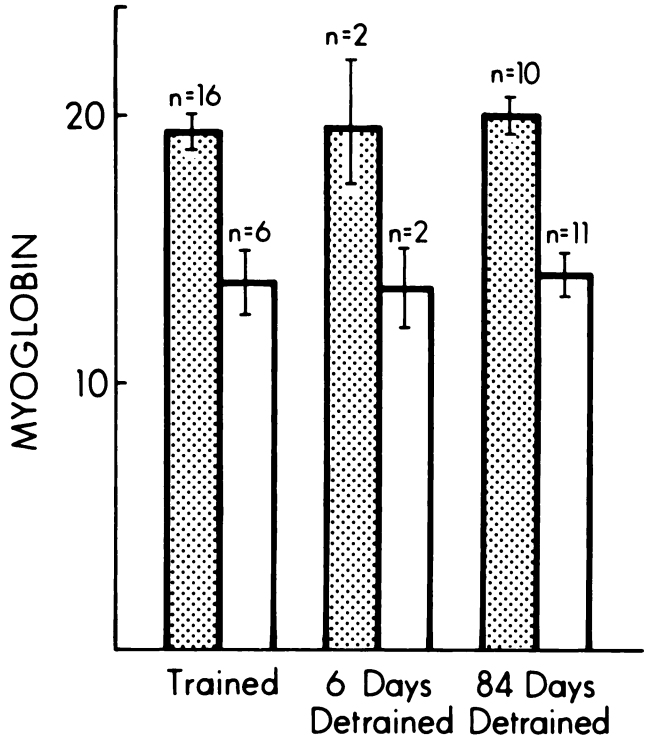
Figure 7. Citrate synthase activity (mol/kg dry wt/hr) and myoglobin content (mg/g dry wt) in the same fibers as in Figure 1.

Until recently, the association between myoglobin content and oxidative capacity in individual human fibers was based on qualitative staining techniques. There were no differences observed in the amount or pattern of staining for myoglobin among human fibers using a fluorescent antibody technique (17). Presumably the technique was not sensitive enough to detect modest quantitative differences. With the benzidine–peroxidase reaction for heme-containing substances, considerably more myoglobin was estimated in the type I fibers than in type II for rat, rabbit, chicken, cat, and human muscle (7). The staining reaction is not specific for myoglobin, therefore, it is possible that myoglobin differences had been overestimated.

Biochemical studies on rodent muscles that consist mainly of type IIB fibers show very low levels of myoglobin and the fibers can be considered truly "white," whereas muscles containing predominantly either type I or IIA fibers have high myoglobin levels and are truly "red" (13,22). The human muscles studied here are known to contain comparable numbers of type IIA and IIB fibers. However, the present quantitative results show that within the type II group there were no fibers with notably low myoglobin and therefore no indication of a fast-twitch white subgroup. In fact, the type II fibers as a whole contain at least two-thirds as much myoglobin as the type I fibers. Clearly, in the case of human muscle, the designation of "fast-twitch white" is not appropriate.

Myoglobin concentrations are shown to vary widely among the three different human muscles studied. Similar differences apparently also exist among human muscles of the same kind. Variations of the magnitude reported in this study were also reported for human quadriceps femoris muscles in whole muscle homogenates (15,21). In addition, Jansson and Sylven (14) studied vastus lateralis muscles from four different people.

Figure 8. Myoglobin concentration (mg/g dry wt ± SD) in type I (stippled columns) and type II fibers (open columns) of trained athlete, at peak training and after 6 and 84 days of detraining. *n*, number of fiber samples.



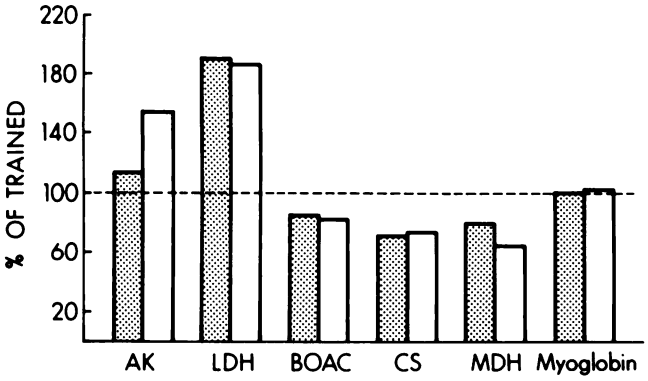


Figure 9. The percent of change in enzyme activities and myoglobin in the same type I (stippled columns) and type II (open columns) fibers of vastus lateralis muscle of the athlete after 84 days of detraining.

They found that myoglobin differences between individual muscles were as great as differences between groups of pooled type I and II fibers from the same muscle.

Detraining of the highly trained athlete resulted in marked decreases in energy-related enzymes, as demonstrated recently (2) in agreement with previous studies (8). Despite the enzymatic changes, myoglobin content was not affected in either fiber group of the vastus lateralis muscle by detraining. A positive correlation with the oxidative enzymes (muscles C and D in Figures 5–7) and negative correlation with the glycolytic enzyme (muscles C and D in Figure 2) remained. It is not possible to determine from this study whether type-specific myoglobin increases occur with training, as shown for rats (9,10), because there is no normal data on the athlete studied. However, the failure in this case of myoglobin to change in either fiber type after 12 weeks of detraining suggests no myoglobin adaptation with training. Moreover, this idea is supported by two recent studies using mixed-fiber homogenates of human muscle samples by Coyle et al. (5) and Svedenhag et al. (25).

The overall conclusion of this study is that in contrast to some species, human muscle fibers do not show great differences in myoglobin levels, the same being true for oxidative enzymes. Furthermore, there is evidence to suggest that a change in training condition which leads to changes in oxidative enzymes does not affect myoglobin levels of the same cells.

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