EXPERIMENT K-6-07

METABOLIC AND MORPHOLOGIC PROPERTIES OF MUSCLE FIBERS AFTER SPACEFLIGHT

Principal Investigator:

R. Edgerton
Department of Kinesiology
University of California
Los Angeles, California 90024-1568

Co-Investigators:

B. Miu
Thomas P. Martin
R. Roy
University of California
Los Angeles, California 90024

J. Marini
J.J. Leger
Unite' de Recherches Neurobiologique
INSERM U6
230 Bd Ste Marguerite
13009 Marseille, France

V. Oganov
E. Ilyina-Kakueva
Institute of Biomedical Problems
Moscow, USSR

INTRODUCTION

Based on previous Cosmos biosatellite and space shuttle (SL-3) flights, it is apparent that a variety of biochemical and physiological properties of rat skeletal muscle are altered following 5-22 days of exposure to microgravity (4,5,8,13,15a,18,19,20,22,23,24,29,30). Since these studies have been based primarily on the analysis of whole muscle properties and given the potential difference in the response of muscle fibers differing in alkaline adenosine triphosphatase type (ATPase) and size, the adaptation to space flight of single muscle fibers were studied. The purposes of this study were 1) to define the size and metabolic and responses of single fibers to space flight and 2) to determine the specificity of these responses to the muscle and the myosin type and size of its fibers. The present findings also permit a comparison with similar data obtained from the ground-based experimental model hindlimb suspension, which is intended to simulate the conditions of space flight (7,9,10,28). Previous studies using hindlimb suspension suggest that the magnitude of the adaptive response of fibers is dependent on the muscle and the ATPase type properties of its fibers (7,9,28). The present data suggest that some fibers acquired a higher glycolytic potential (alpha-glycerolphosphate dehydrogenase; GPD) and the rate at which they can hydrolyze ATP is increased. Further, the potential of the tricarboxylic acid cycle, as indicated by succinate dehydrogenase activity (SDH), was maintained or elevated depending on the muscle and ATPase type. These data suggest a shift in the metabolic profile of the fibers to that consistent with the fast oxidative-glycolytic profile defined by Peter et al. (2,1). Also, in the present data the degree of atrophy in the flight muscles depended more on the muscle and the region of the muscle than on fiber type as defined by ATPase staining or the immunohistochemical properties. The similarities between these data those from a previous space shuttle flight (SL-3) and those of hindlimb suspension are striking (7,9,10,28).

METHODS

Five male rats, body weight = 303.2 $\pm$ 2.4 ($\bar{X}$ + SE), flown on Cosmos 1887 for 12.5 days were studied. An additional five rats, body weight = 349.0 $\pm$ 5.8, were maintained under identical ground based conditions (cage size, temperature, lighting, and food and water availability) for the duration of the mission and acted as a synchronous control. Details of the experimental protocol related to flight conditions are described elsewhere. Ground based control rats were killed on a similar time schedule as the flight rats. From each rat, the left soleus (SOL) and the medial gastrocnemius (MG) muscles were resected, weighed. The muscle was mounted on cork and frozen in freon cooled in liquid nitrogen. All samples were maintained at -70^{o}C until analyzed. Frozen serial tissue sections (10mm thick) were cut at -20^{o}C in a cryostat. Sections were prepared for the qualitative histochemical determination of alkaline (pH 8.8) myofibrillar ATPase staining density in a population of fibers from each muscle according to the modification of Brooke and Kaiser (2) as described by Nwoye et al (17). Fiber cross-sectional areas also were determined from these ATPase-stained sections. The same fibers from serial sections were subsequently prepared for the determination of SDH and GPD activity as described by Martin et al. (16). Frozen sections of the Sol were also reacted to antibodies for slow and fast myosin. Fasicles of fibers free of tissue artifact and considered visually to be representative of the tissue section were chosen for analyses. A computer assisted image analysis system was used to quantify the reaction product based on the rate of change of optical density (OD) for each fiber. The rate of staining (OD/min) was directly proportional to the enzyme activity as reported by Martin et al. (3,16). The hardware and software components of this system have been described previously (2,4,16).

RESULTS

Cross Section Area

Mean wet muscle weights of the flight and control rats were 130.4 and 153.4g, a 15% decrease (Fig. 1). Muscle fiber cross sectional area (CSA) in the Sol was about 50% lower in flight than control rats. The percent difference in CSA in the Sol between flight and control was similar in light and dark ATPase as well as those that stained moderately with ATPase. The mean wet weights of the flight and control MG were 634.9 and 754.9g, a 16% decrease. The mean CSA of the light and dark ATPase fibers of the deep MG were 17 and 28% smaller than control while the dark ATPase fibers of the superficial MG were 15% smaller (Fig. 1). Based on the population distribution of each of the ATPase types in the Sol and MG, it is clear that a general downward shift in the CSA of the population of fiber occurred (Fig 2 and 3) as opposed to an apparent shift in any portion of the population

Succinate Dehydrogenase Activity

Mean SDH activity was unchanged for each of the ATPase categories in the Sol (Fig. 4). However examination of the population distribution for SDH activity suggests a slight shift toward higher activities in the light ATPase fiber and a slight downward shift in the dark ATPase fibers (Fig.5). To reflect the total amount of enzyme that may have changed per muscle fiber, the product of activity and CSA were calculated. These results show that the net amount of the SDH enzyme, assuming no change in specific enzyme activity, was significantly lower than control in each ATPase type of the Sol in the flight rats (Fig.4).

In the MG the mean SDH activity remained at control levels in the flight rats for each ATPase type. The population distributions also suggest that no changes occurred in SDH activity (Fig. 7). Further, there was no effect of flight on the integrated SDH activity in any ATPase type in the MG (Fig. 6).

Glycerolphosphate Dehydrogenase Activity

In the Sol, mean GPD activity was elevated significantly in the dark, but not in light or intermediate ATPase types in the flight rats (Fig.8). In control and flight rats the dark ATPase fibers of the Sol had higher GPD activity than the light ATPase fiber. Based on the population distribution it can be seen that values of near zero activity in the dark ATPase fibers were rare in the flight, but common in the control rats (Fig. 9).

In the MG there were no GPD activity changes due to flight in either of the ATPase types in either the deep or superficial regions (Fig. 10). In control rats the GPD activities of each ATPase type differed between the Sol and MG. For example, GPD of the light ATPase fiber was lower in the MG than the Sol while in the dark ATPase fiber GPD was lower in the Sol than the MG. The dark ATPase fiber of the superficial MG had the highest GPD activity of any fiber. In both control and flight rats, GPD activity had a skewed distribution with a predominence of low values in the deep MG while the population distribution was normal in the superficial MG (Fig.11). There was a strong hint of a shift toward higher GPD values in the superficial MG of flight compared to control, although the mean was not significantly different.

The integrated GPD (GPD activity x CSA) of each ATPase type in both muscles was similar in the control and flight rats (Fig. 8 and 10), suggesting no net change in the amount of this enzyme per fiber. This is quite remarkable, particularly, for the Sol given the marked atrophy that occurred in this muscle.

Enzyme Activity Ratios

In the Sol the GPD:SDH activity ratio was elevated in the dark ATPase fiber, but not in the light or intermediate ones. In control rats, the GPD;SDH ratio was higher in the light than dark ATPase fibers. In the flight rats, the dark ATPase fibers had the higher ratio, thus reflecting the large increase due to flight on this one ATPase type.

In the MG the GPD:SDH activity ratios were unaffected by flight. The magnitude of the ratios were light ATPase,deep < dark ATPase, deep < dark ATPase, superficial, with these mean ratios differing by 40-fold. The mean GPD:SDH ratios were about 10-fold higher in the MG than the Sol (Fig.12).

Immunohistochemistry and Myosin ATPase Staining

Immunohistochemical reactions were completed in the Sol of 4 control and 4 flight rats (Table 1). One antibody was used to label slow myosin and one to label fast myosin. These results were then compared with the qualitative myosin ATPase staining reaction as well as to SDH and GPD activities and finally fiber size (Table 2). Based on these antibody reactions, fibers were separated into Type I (reacted only to slow antibody), Type "IIc" (reacted to both slow and fast myosin antibodies) and IIa+b (reacted only to fast myosin antibody).Using this approach 77.9% of the Sol fibers were Type I in the control rats compared to 60.2% in flight rats (Table 1). There were 8.0% "IIc" fibers in control and 30.9% in flight rats. The remaining percent of Type II(a+b) fiber in the control was 14.0% and in the flight rats, 9.0%.

Based on myosin ATPase staining at a basic pH, 15.9% of the fibers were darkly stained in control and 20.3% in flight rats. It appears that the immunohistochemically defined "IIc" fibers in control rats were consistently categorized as darkly stained with myosin ATPase, base (Table 2). However, in the flight rats it appears that the newly occurring "IIc" fibers did not fall consistently into either the light or dark ATPase category (Table 1 and 2). There was about a 4-fold greater proportion of "IIc" fiber (8.0% vs. 30.9%) in flight than control rats while the difference in proportion of dark ATPase fibers suggested only approximately a 28% increase (15.9% vs. 20.3%). In the flight rats it appears that there was a reduction in percent type I, increase in "IIc" and reduction in percent IIa+b (Table 1).

In a select group of fibers identified as reacting to slow and fast antibodies from 2 control and 2 flight rats, a profile of other features were compared at the single fiber level so as to determine the degree to which they could be associated with other quantitative measures (Table 2). The profiles of individual IIc fibers in the two control rats (S6, n=2 and S7, n=6) more closely match the dark ATPase profile with respect to myosin ATPase stain, SDH activity, and CSA. In two flight rats (F8, n=7, and F9, n=9) the "IIc" fibers could not be clearly associated with either ATPase type and the related enzyme properties. In the flight rats it is clear that some of the "IIc" fibers were categorized as light and some dark myosin ATPase. The mean profiles of light and dark ATPase fiber in Table 2 were derived from the mean of all of the fibers analyzed for rats S7, F8 and F9.

DISCUSSION

Data from previous Cosmos flights have demonstrated that a space flight of 5-22 days results in muscular atrophy (4,5,8,13,18,19,20,22,23,29,30). The decreased muscle mass observed in the present study are consistent with these reports. While both the Sol and MG of the flight animals showed atrophy based on fiber CSA,the degree of atrophy was different among the muscles, e.g., the SOL being the more than twice as atrophic as the MG. Further, the atrophy in the super region of the MG was less than in the deep region. This differential response among muscles and muscle regions to adaptive perturbations has been a common finding in many studies (7) and is similar to the responses to hindlimb suspension (8,28). A direct comparison of the fiber size changes in the

7-day shuttle flight (SL-3) and the present 12.5 day Cosmos 1887 flight can be made since the same muscles were studied using the same procedures in the same laboratory. The overall CSA of the Sol of SL-3 rats suggested 35% atrophy compared to 45% in the present Cosmos data. Similar comparisons of the MG suggests 16% atrophy occurred in the SL-3 rats and 20% in the Cosmos 1887 rats.

In addition to the specificity of the effect of space flight on certain muscles and fiber types, there seems to have been an unequal effect of space flight on various muscle proteins. For example, the cross-sectional areas of both light and dark ATPase fibers in the flight SOL and MG were reduced while the SDH and GPD activities in these fibers remained the same or were enhanced. Identical conclusions were made from similar studies of the 7-day flight SL-3 rats (15a). A preferential loss of muscle volume (and probably contractile protein) relative to metabolic enzymes in the muscles may account for the fact that SDH and GPD activity was essentially maintained after 12.5 days of flight. If the net rate of degradation and synthesis of SDH or GPD was unchanged per fiber, or reduced by a lesser amount relative to other proteins, and assuming no change in the specific activity, enzymatic activity would be elevated proportionate to the reduction in fiber volume. Stated more simply, if the number of SDH or GPD molecules per fiber had remained the same or decreased less than fiber volume, the enzyme activity would be elevated. Based on this concept, the average light or dark ATPase fibers of the SOL may have lost about 40% of the total amount of the SDH enzyme per fiber (product of CSA and SDH activity = integrated OD) even though the SDH activity, which reflects its concentration, remained virtually the same as control levels. Using this same rationale, the low ATPase fibers in the flight MG had control levels of SDH per fiber. Similar calculations were made for GPD among the different fiber types and muscles. These results suggest no net changes in the number of GPD enzyme molecules per fiber as a result of flight. This was true for all ATPase types in the Sol and MG. The integrated enzyme activities in the present study were also similar to that observed in the SL-3 rats. In the Sol, it does appear, however that the loss of SDH per fiber was about twice as great in the Cosmos than the SL-3 rats. Obviously, an important question in considering the effects of long-term space flight is whether there is some point in time at which these proteins and the cell volume reach a homeostatic state.

The quantitative histochemical assessment of single fibers enzyme activities after a 12.5 day flight study showed that oxidative and glycolytic metabolic capacity essentially is maintained or enhanced following space flight just as has been reported in the same muscles after a 7 day flight It is interesting to note that based on qualitative visual assessments of oxidative enzymes in previous studies or rats flown on a Cosmos biosatellite, the oxidative capacity of the SOL appeared to have been reduced (13). However, the glycolytic potential of the SOL was reported to have increased (13). This latter but not the former observation has now been confirmed using quantitative analyses after two space flight studies. Also, a shift in the SOL muscle LDH isoform profile toward the predominant M-isoform has been reported in flight animals (22). These glycolytic enzyme data are consistent with the present study in that slow muscles (SOL) tend to develop characteristic of fast muscles (which also have a high relative glycolytic potential). The GPD activity data suggest that some flight muscles may have an elevated capacity to utilize carbohydrate derived carbon sources. It is of interest to note that the glycogen content of both the SOL and EDL was elevated in SL-3 flight rats relative to control (11) even though the rats had remained at 1G for about 11 hours after the 7 days at 0G.

In SL-3 rats there was an increase in the biochemically assayed myofibrillar ATPase activity of a homogenate of the flight SOL. This was consistent with the increase in the percentage of fibers that stained darkly with ATPase and the enhanced GPD activity. These two enzymes have been shown to be highly correlated and to remain so in the face of marked alterations in the properties of a muscle as occurs in chronically spinalized animals (25). The increase in myofibrillar ATPase activity measured in the homogenate of the SL-3 rat Sol and the higher proportion of dark ATPase fibers in this muscle are consistent with the report of an increase in the fast myosin light chain isozymes in the SOL of a previous Cosmos study (30). However, there are other data from a

Cosmos flight in which myosin ATPase activity in the SOL of rats was reduced (13). This latter finding appears to have been an anomaly. The conversion of light ATPase staining fibers to dark staining in SL-3 rats did not completely reflect the quantitative myofibrillar ATPase changes reported (15a).

Based on the present results, the significance of the previously reported increase in percentage of dark ATPase fibers has additional significance. For example, in the present study there was an increase in percent dark ATPase fibers using a basic pH as there was in SL-3 rats. However, this may underestimate the number of fibers that were induced to synthesize fast myosin. Based on immunohistochemical identification of slow and fast myosin types 31% of the Sol fibers contained both slow and fast myosin types. In control rats, only 8% had this property. However, in flight rats, the percentage of slow fibers based on myosin ATPase, basic pH, was overestimated when compared to the percentage that reacted positively to only a slow antibody (80% vs. 60%). It appears that about 10% of the fibers in the Sol of flight rats had begun to synthesize fast myosin, but was not reflected in an enhanced myosin ATPase staining reaction. This is as it might be expected in that the more sensitive indication of fast myosin would be expected to be immunological. Fibers that stain darkly at an acid and basic pH when staining for myosin ATPase have been call IIc fibers. In the present work when there was a immunohistochemical identification of both myosin types we have labeled them "IIc".

Although it appears that most of the nearly acquired "IIc"fibers in the Sol of the flight rats develop from light ATPase fibers (commonly called Type I), it is possible that some of the dark ATPase (Type II) fibers that normally would have reacted only to a fast myosin antibody, also reacted positive to a slow myosin antibody after flight. When comparing each of the parameters studied with respect to their ATPase type the "IIc" fibers resembled the dark ATPase fibers more than the light ATPase fibers Table 2. This was the case in comparing the SDH and GPD activities, as well as CSA.

The interrelationship between ATPase, SDH and GPD activity in the fibers of the flight muscles suggests that the metabolic profiles found in normal rats also occur in fibers following exposure to microgravity. In the SOL more fibers stained darkly with the ATPase stain in the flight than control rats. In conjunction, it appears that these same fibers maintained or increased their GPD activity while maintaining their SDH activity. As a result, a greater percentage of fibers in these muscles could be categorized as fast oxidative-glycolytic. This shift in metabolic profile apparently occurred at the expense of slow oxidative fibers.

The explanation of why muscles atrophy in space flight and why the effect of space flight differs among muscles has not been defined clearly. Based on carcass composition and the weights of various vital organs, the rats flown aboard the space shuttle mission, SL-3, were healthy and experienced minimal stress (8). It is unlikely that the changes observed in the present study can be attributed to factors associated with increased levels of glucocorticoids. However, a general loss of muscle mass is consistent with the decrease in the growth hormone observed in the pituitary gland of SL-3 rats (12). Also, the in vivo release of growth hormone from pituitary gland cells decreased in the SL-3 animals (12).

The present study demonstrates that the general capability of skeletal muscle to maintain its proteins decreases rapidly in response to space flight. The present findings suggest further that the magnitude of enzymatic and cell volumes changes in response to space flight depend on several factors including the muscle and its fiber type composition. It appears that in order to associate physiological relevance to the observed enzymatic changes, cell volume should be considered also. Although it remains unclear as to the stimulus, or lack of stimulus, that triggers the rapid changes in muscle proteins in response to space flight, ground-based models of muscle atrophy suggest that the reduction in mechanical loading of muscle may be more important than the total amount of activation over a 24-hr period (1).

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TABLE 1. FIBER TYPE POPULATIONS BASED ON IMMUNOHISTOCHEMISTRY AND MYOSIN ATPASE

	Immunohistochemistry				Myosin ATPase (pH 8.8)		
Animal	I	IIc	IIa+b	Total	Light	Dark	Total
S6	1326	49	257	1632	1712	397	2109
%	**81.3**	**3.0**	**15.7**		**81.2**	**18.8**	
S7	1544	152	160	1856	1612	220	1832
%	**83.2**	**8.2**	**8.6**		**88.0**	**12**	
S8	865	192	188	1245	1555	398	1953
%	**69.5**	**15.4**	**15.1**		**79.6**	**20.4**	
S9	1249	94	267	1610	1619	346	1965
%	**77.6**	**5.8**	**16.6**		**82.4**	**17.6**	
S10	-	-	-	-	1133	132	1237
%					**89.3**	**10.7**	
Mean							
%	**77.9**	**8.0**	**14.0**	**100**	**84.1**	**15.9**	**100**
F6	1065	522	160	1747	1393	307	1700
%	**61.0**	**29.9**		**9.2**	**81.9**	**18.1**	
F7	-	-	-	-	1764	494	2258
%					**78.1**	**21.9**	
F8	1050	440	157	1647	1498	382	1880
%	**63.8**	**26.7**	**9.5**		**79.7**	**20.3**	
F9	1178	542	125	1845	1223	339	1568
%	**63.8**	**29.4**	**6.8**		**78.3**	**21.7**	
F10	582	418	118	1118	1474	362	1836
%	**52.1**	**37.4**	**10.6**		**80.3**	**19.7**	
Mean							
%	**60.2**	**30.9**	**9.0**	**100**	**79.7**	**20.3**	**100**

TABLE 2: CHARACTERISTICS OF "IIC" FIBERS IDENTIFED IMMUNOHISTOCHEMICALLY

Rat	Fiber Identification	*Antibody S - F	Myosin Type	SDH (OD/Min x 10^{-4})	Area (μm^2)	GPD (OD/min x 10^{-4})
S6	38	+ +	dark	629	4067	0**
	49	+ +	dark	610	4554	1
S7	19	+ +	dark	711	4184	18
	8	+ +	light	485	3295	26
	65	+ +	interm	504	2855	16
	67	+ +	dark	559	3372	15
	55	+ +	dark	582	4592	17
	24	+ +	dark	516	3423	19
***Overall Mean Profile, S7		+ -	Light	310	5048	6.9
		- +	Dark	548	4006	5.9
F8	1	+ +	dark	428	1741	14
	12	+ +	light	330	1420	10
	26	+ +	dark	403	1519	15
	38	+ +	light	343	1457	10
	77	+ +	dark	363	1593	18
	48	+ +	dark	353	1915	11
	23	+ +	dark	468	1115	19
***Overall Mean Profile, F8		+ -	Light	258	2236	5
		- +	Dark	444	2107	19
F9	75	+ +	light	449	2088	10
	80	+ +	dark	515	2263	8
	54	+ +	dark	485	1108	28
	66	+ +	dark	393	2274	21
	77	+ +	dark	485	2418	33
	68	+ +	light	419	2387	13
	25	+ +	light	338	4283	15
	27	+ +	light	434	3223	26
	40	+ +	dark	460	1868	25
***Overall Mean Profile, F9		+ -	Light	344	2771	8
		- +	Dark	502	2170	28

*Positive reaction to slow (+) or fast (+) antibody
**GPD mean of population of fibers were also unusally low.
***Means based on ATPase type of population of S7,F8, or F9.

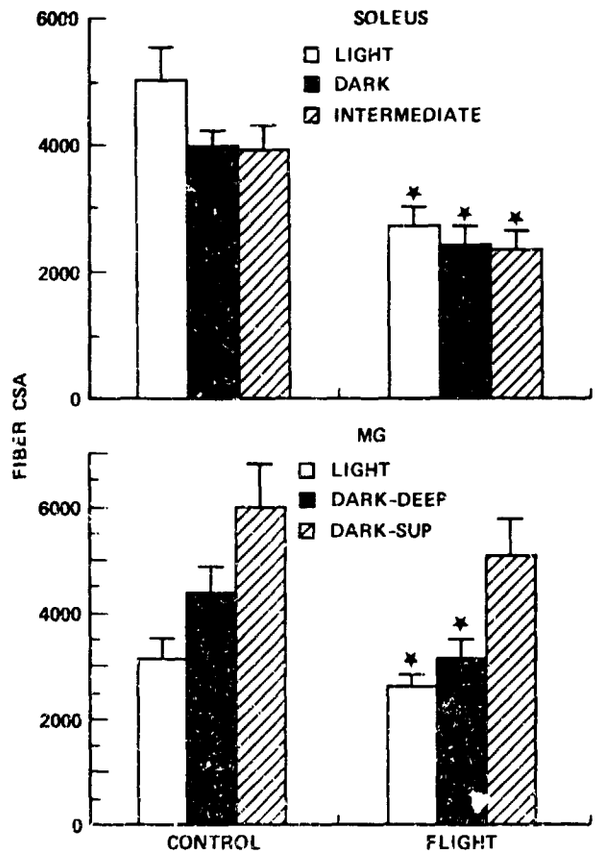

Figure 1. Mean fiber cross-sectional area (CSA, μm^2) of light, dark and intermediate ATPase fibers of the Sol and MG of control and flight rats Vertical bars are SEM. Light and dark ATPase fibers of the deep and dark fibers of the superficial region of the MG are illustrated. *, significant difference between control and flight. $p < 0.05$.

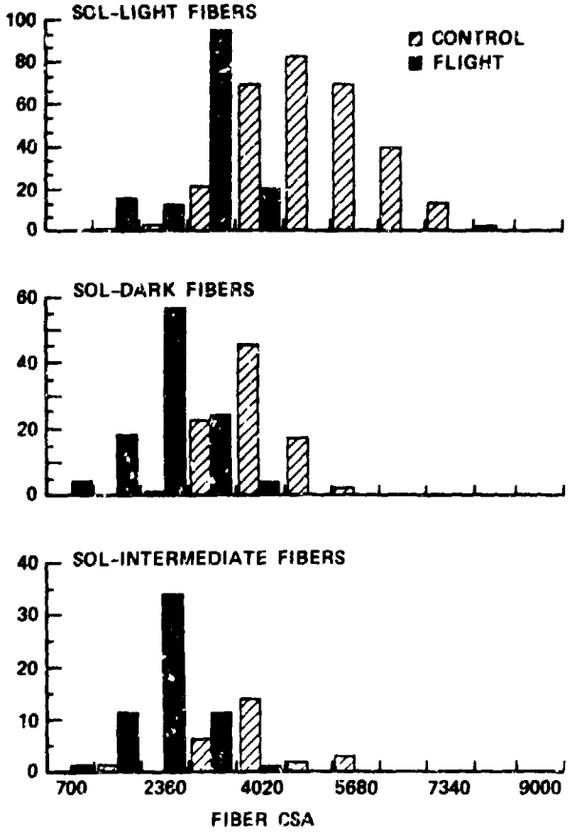

Figure 2. Frequency distributions of fiber cross-sectional area (CSA, μm^2) for light, dark and intermediate ATPase fibers of the Sol of control and flight rats.

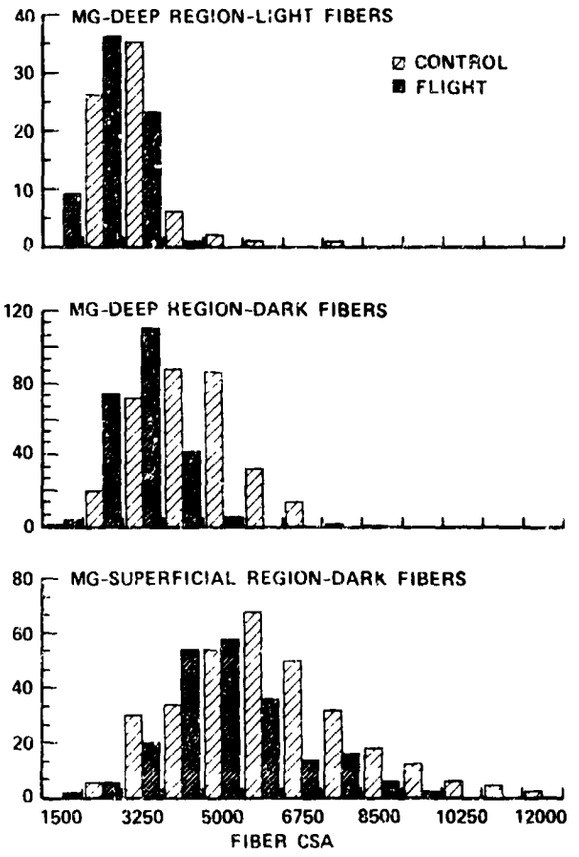

Figure 3. Frequency distributions of fiber cross-sectional area (CSA, μm^2) for light and dark ATPase fibers in the deep and dark ATPase fibers in the superficial region of MG of control and flight rats.

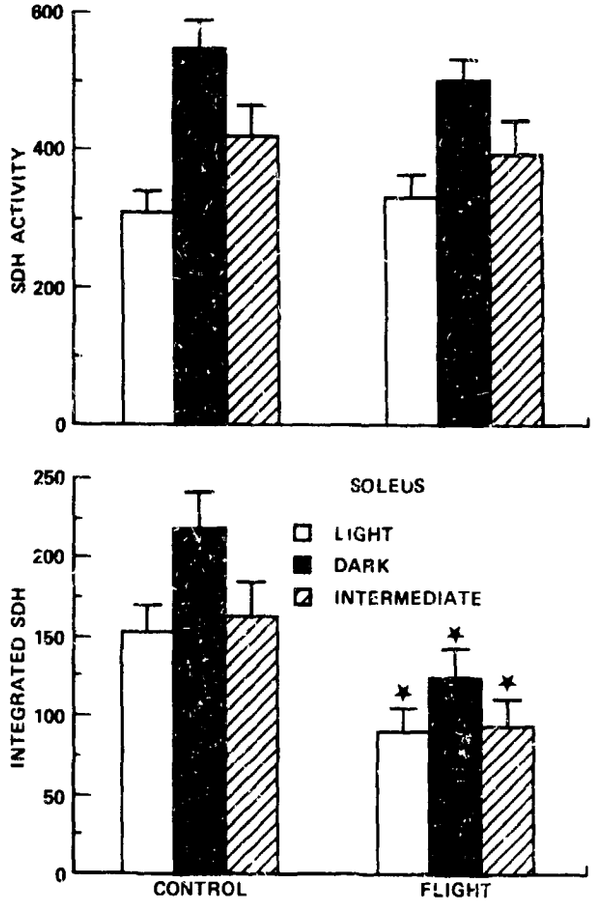

Figure 4. The mean ± SEM of SDH activity (OD/min x 10^{-4}) and integrated SDH (CSA x OD/min) for light, dark and intermediate ATPase fibers of the Sol of control and flight rats. *, $p < 0.05$.

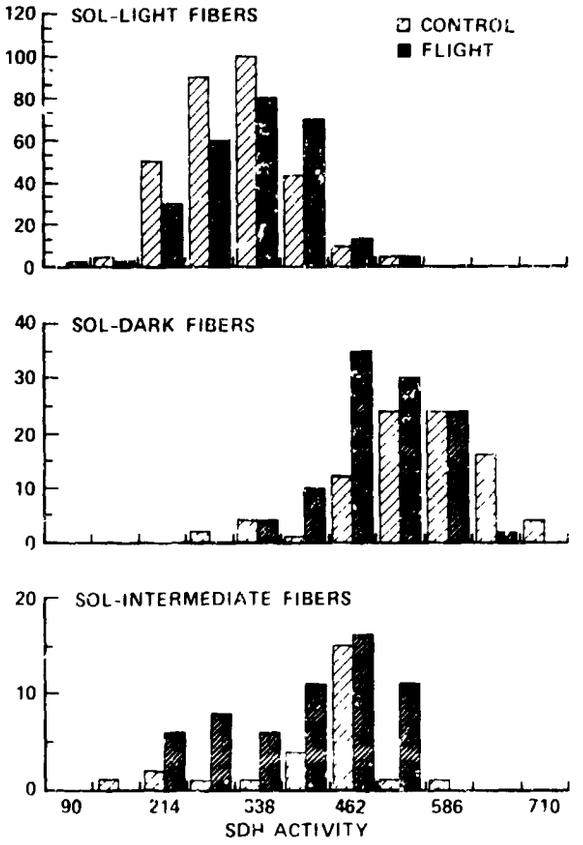

Figure 5. Freq' ency distributions of SDH activity (OD/min x 10^{-4}) for light, dark and intermediate ATPase fibers of the Sol of control and flight rats.

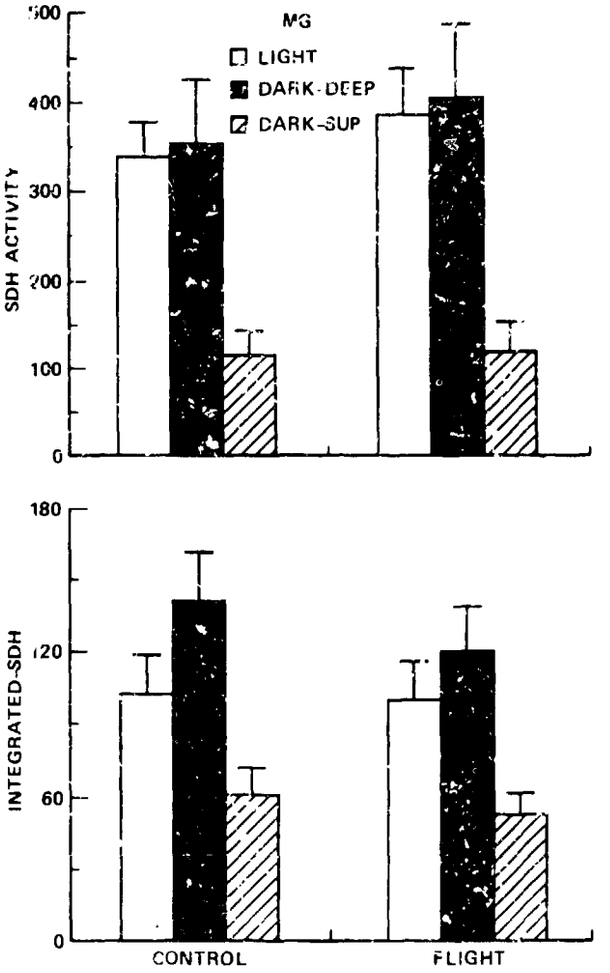

Figure 6. The mean ± SEM of SDH activity (OD/min x10^{-4}) and integrated SDH (CSA x OD/min) for light and dark ATPase fibers in the deep region and dark ATPase fibers of the superficial region of the MG of control and flight rats.

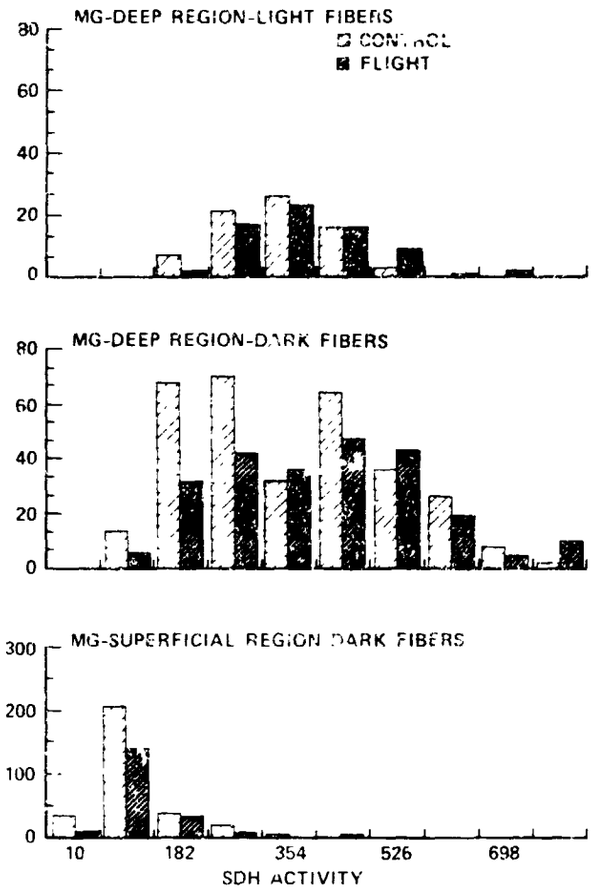

Figure 7. Frequency distributions of SDH activity (OD/min x10^{-4}) for light and dark ATPase fibers of the deep and dark ATPase fibers of the superficial MG of control and flight rats.

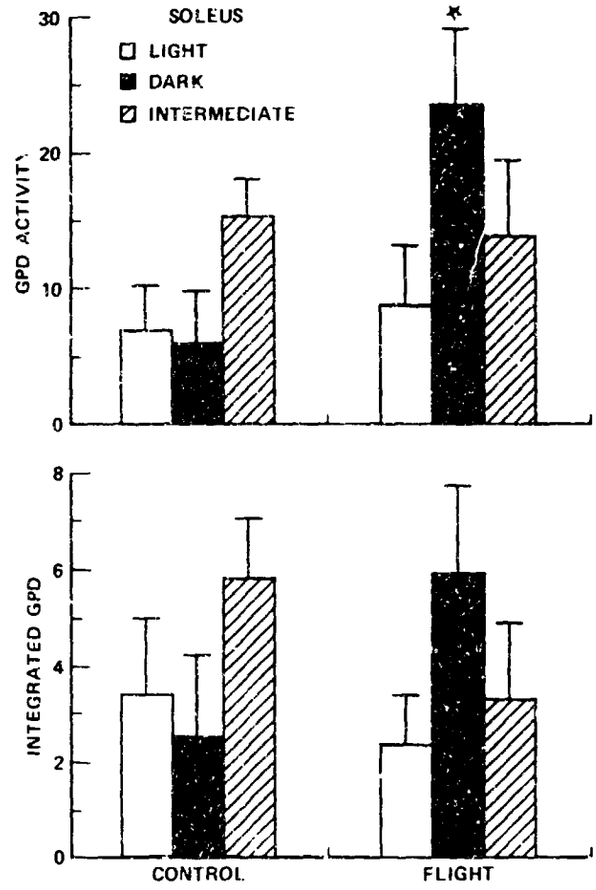

Figure 8. The mean ± SEM of GPD activity (OD/min x 10^{-4}) and integrated GPD (CSA x OD/min) for light, dark and intermediate ATPase fibers of the Sol of control and flight rats. *, $p < 0.05$.

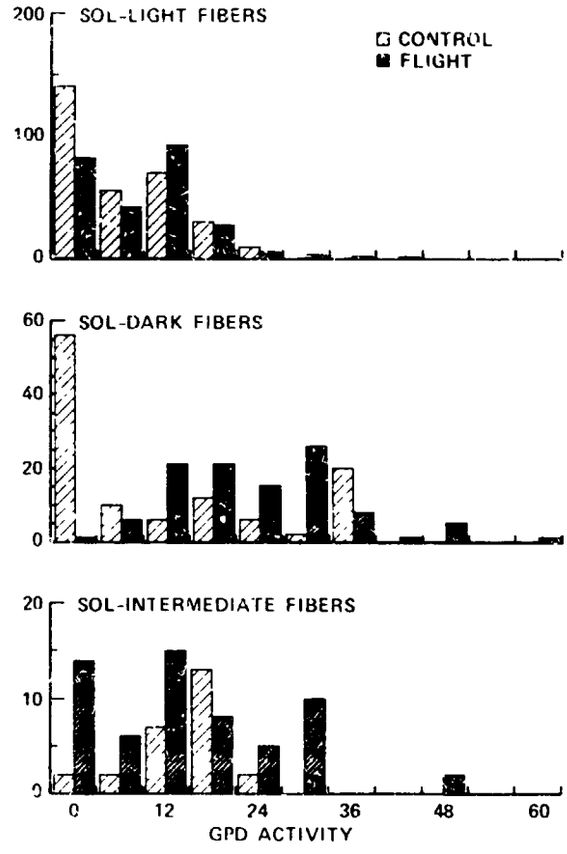

Figure 9. Frequency distributions of GPD activities (OD/min x10^{-4}) for light, dark and intermediate ATPase fibers of Sol of control and flight rats.

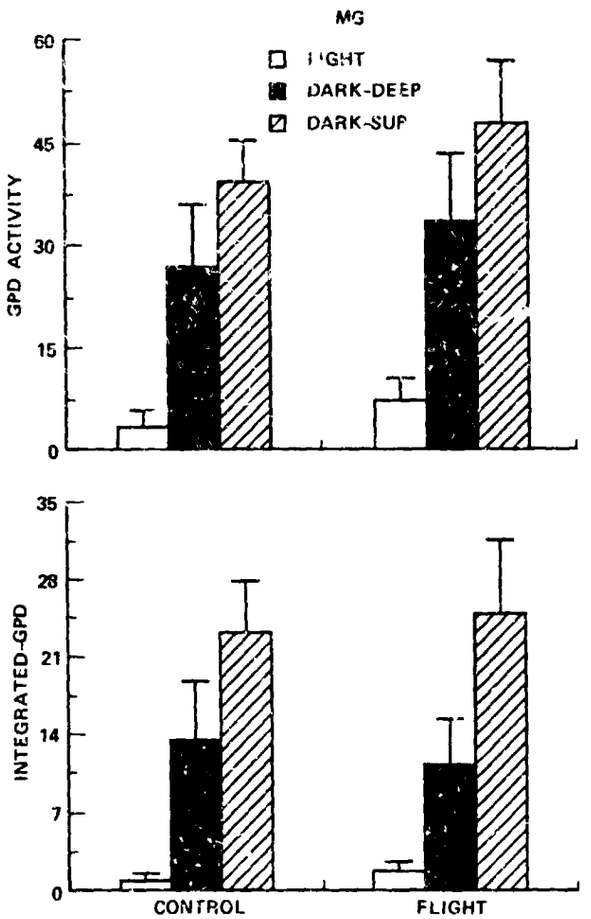

Figure 10. The mean ± SEM of GPD activity (OD/min x 10^{-4}) and integrated GPD (CSA x OD/min) for light and dark ATPase fibers of the deep and dark ATPase fibers of the superficial MG of control and flight rats.

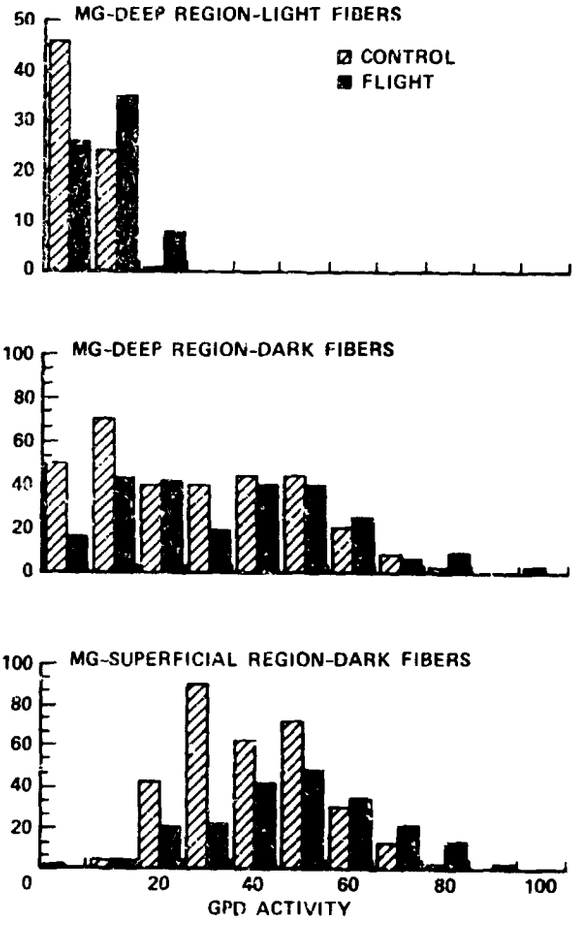

Figure 11. Frequency distributions of GPD activities (OD/min x 10^{-4}) for dark and light ATPase fibers of the deep and dark fibers of the superficial region of control and flight rats.

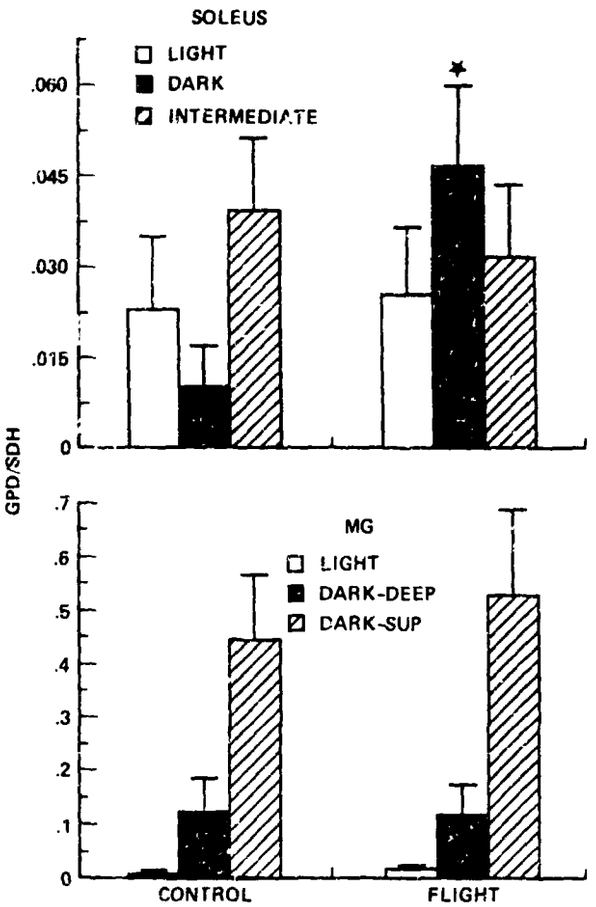

Figure 12. The mean ± SEM of GPD/SDH for light, dark and intermediate ATPase fibers of the Sol, and light and dark ATPase fibers of the deep region and dark ATPase fibers of the superficial region of the MG of control and flight rats. *, $p < 0.05$.