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Final Technical Report Contract No. NAG2-212 Entitled "Alterations in Skeletal Muscle With Disuse Atrophy: The Effects of Countermeasures". P.I. Robert H. Fitts, Marquette University, Milwaukee, WI.

This report presents a summary of the accomplishments for contract No. NAG 2-212, entitled "Alterations in Skeletal Muscle With Disuse Atrophy: The Effects of Countermeasures." The specific aims of this project concerned three general areas: (1) studies on the contractile function of single skinned fibers designed to determine the time course and cellular basis of the hindlimb suspension (HS) induced increase in fiber Vo (maximal shortening velocity), and the decrease in peak tension (Po); (2) studies designed to understand the effect of HS on single fiber substrate utilization during contractile activity, and how if at all such changes contribute to the increased muscle fatigue associated with HS; and (3) studies evaluating the effectiveness of standing and ladder climbing as countermeasures to the deleterious effects of HS. We had also proposed to begin studies examining the effects of HS on excitation-contraction coupling using the voltage clamped single cut fiber. Due to insufficient funds ($58,200/yr total costs, direct and indirect), we were unable to fund the support staff or supplies required of these labor intensive studies. However, we have constructed all of the necessary equipment, and are currently conducting preliminary studies on T-tubular charge movement. A list of publications from this contract is included at the end of this report. In the following paragraphs we will review the major accomplishments for each of the three objectives listed above.

1. Functional Studies on the Single Skinned Fiber.

Our recently published time course studies clearly showed 1 to 3 wk of HS to induce a progressive decline in fiber diameter (14, 22, and 42%, respectively) and peak force (42, 48, and 72%, respectively) in the slow type I fibers of the soleus (3-5). Additionally, HS increase the fast type IIa population in the soleus from a control value of 4% to 29% by 3 wk HS (3). Because the SDS gel system used did not separate the type IIa from the type IIx myosin heavy chain (MHC), the possibility exists that some of the fast fibers were type IIx. However, the majority of fibers following 1, 2, and 3 wk HS showed an increased Vo with no change in the myosin isozyme. A question of considerable importance is what triggered the increased Vo in this population of fibers? One possibility was that the small fibers segments (approximately 2 mm) contained fast myosin but in such small amounts that it went undetected on our SDS gels or that the increase in fiber Vo was caused by changes in the myosin light chain (MLC) profile. Consequently, one objective was to categorically demonstrate that fiber Vo indeed increased without an alteration in the myosin isozyme. We isolated 200-300 fibers from soleus muscles of control, 1, and 3 wk HS animals, and loaded the entire fiber (6-7 mm) on 12% SDS gels. The gels were analyzed for fiber type distribution (slow vs. fast), and scanned for the determination of MLC stoichiometry. In a subset of fibers isolated from the soleus of control and 2 wk HS animals, fiber Vo and the myosin heavy and light chain (MHC & MLC) profiles were determined on the same fiber. In these experiments in addition to the 12% gel, a 3.5% (w/v) acryamide stacking gel with 5% separating gel was employed to separate the fast

MHC's into type IIa, IIx and IIb. The fiber type distribution is shown in Table 1 (4). At 1 wk the percent of slow type I fibers had decreased from 82 to 74%, while the number of hybrid fibers (fibers containing both slow and fast myosin) had increased from 10 to 18%. Interestingly, by 3 wk HS the fast fiber percentage had increased to 26%, while the number of hybrid fibers declined to 5%. These data indicate that the increased fast fiber population occurred via fiber transition from slow- to fast-twitch via a hybrid fiber population (slow to hybrid to fast) rather than through a selected drop out of slow fibers and synthase of new fast fibers from satellite cells.

TABLE 1.

	# of Fibers	% Slow	% Fast	% Hybrid
CONTROL	195	82	8	10
1 wk HS	271	74	8	18
3 wk HS	246	69	26	5

Although it is apparent that HS does induce a transformation of fibers from slow-to -fast, a second subpopulation of slow fibers (approximately 70% of the total number of slow fibers) showed an elevated Vo and fiber ATPase activity with no detectable fast myosin (3,4). Since the total number of fast fibers following 3 wk HS was the same whether fiber segments or whole fibers were assayed, the increased Vo is not likely to be due to the presence of undetectable amounts of fast myosin. Changes in the MLC stoichiometry might upregulate the myosin ATPase and thus increase Vo, however, following HS we found no significant change in the LC2s/LC1s + LC2s ratio. Furthermore, there was no correlation between the MLC ratio and the observed increase in fiber Vo (4).

With HS the individual slow fibers of the soleus showed a progressive decline in peak force from the control value of 39.6 ± 0.8 to 23.0 ± 0.8, 20.5 ± 1.2, and $10.9 \pm 0.7 \times 10^{-5}$ N after 1, 2, and 3 wk HS, respectively (3). In contrast, the force per cross-sectional area declined in the first wk of HS from 106 ± 2 to 87 ± 2 kN/m^2 and than remained constant through the second and third wk of HS (3). In a recent study, we examined the stiffness of the slow type I soleus fibers (a marker of the number of cross-bridges per CSA) and found it to decline in the first 2 wk of HS from 1.96 ± 0.12 to $0.96 \pm 0.09 \times 10^7$ N/m^2 (5). The decline in peak stiffness (Eo) exceeded that observed for peak force (the Po/Eo ratio increased). Our working hypothesis is that the loss of contractile protein reduces the number of cross-bridges per cross-sectional area and increases the filament lattice spacing. The increased spacing reduces cross-bridge force and stiffness, but P_o/E_o increases because of a quantitatively greater effect on stiffness. We also hypothesize that the increased fiber V_o in the slow type I fibers of the soleus, and right shift in the force-pCa curve are caused by the increased lattice spacing (3,5).

One week of HS resulted in an upward shift of the force-velocity curve, and between 2 and 3 wk the curve shifted further such that V_o was higher than control at all relative loads <45% peak isometric force (4). Peak absolute power output of soleus fibers progressively

decreased through 2 wk of HS but showed no further change at 3 wk. These results suggest that between 2 and 3 wk the HS-induced alterations in the force-velocity relationship act to maintain the power output of single soleus fibers despite a continued reduction in fiber force (4)..

2. Fiber Substrate Utilization and Muscle Fatigue with Contractile Activity.

In previous work, we had shown that HS increased the fatigability of the soleus muscle, and this change occurred without a reduction in krebs cycle enzymes. In this contract, we evaluated whether or not the increased fatigability was caused by an altered tissue blood flow. Although HS produced a decreased hindlimb blood flow relative to the standing position, there was no significant differences in soleus muscle blood flow between the control and 15 d HS groups during standing or treadmill running at 15 m/min (1). Importantly, the HS rats did not show the expected decline in visceral organ blood flow with exercise, and the flow to predominantly fast-twitch hindlimb muscles was somewhat higher than the control. The higher flow to the viscera and fast twitch fibers suggests an apparent reduction in the ability of the sympathetic nervous system to distribute cardiac output after chronic HS (1).More recently, we studied soleus muscle fatigue in situ, and found that HS increased fatigability but had no effect on soleus blood flow (2). Thus we can conclude that the increased fatigability is not caused by a reduced muscle blood flow. Currently, our working hypothesis is that fat oxidation of the slow type I fiber is somehow compromised leading to an increased dependence on carbohydrate metabolism. If true, during contractile activity the individual slow type I fibers of the soleus should deplete muscle glycogen and show higher lactate concentrations. In these experiments, we produced fatigue via electrical stimulation of the soleus in situ, and quick froze the muscle after 0, 2, 5, and 10 min of contractile activity. The muscles were than freeze-dried, and individual single cells isolated and assayed for glycogen, lactate, ATP, and phosphocreatine (PC). The reactions were carried out under oil which allowed all four substrates to be assayed in each fiber studied. Lactate was higher at rest in the cells from the HS animals, and reached significantly higher levels with contractile activity (Figure 1). Consistent with the increased lactate production, we observed a greater glycogen depletion in the soleus fibers from the HS animals (Figure 2).

FIGURE 1.

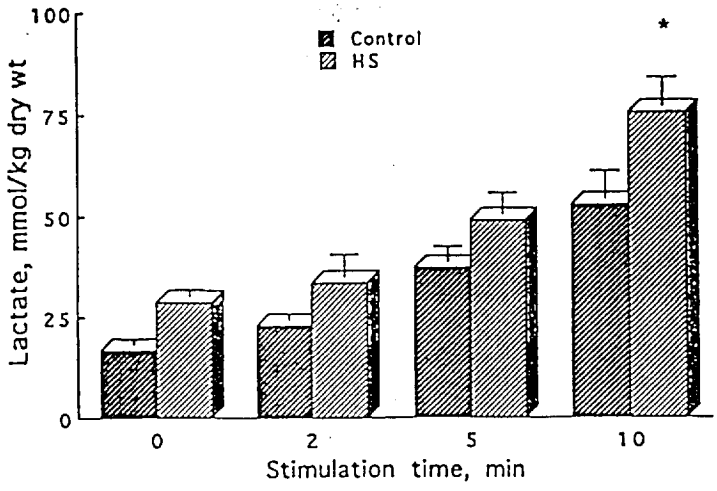
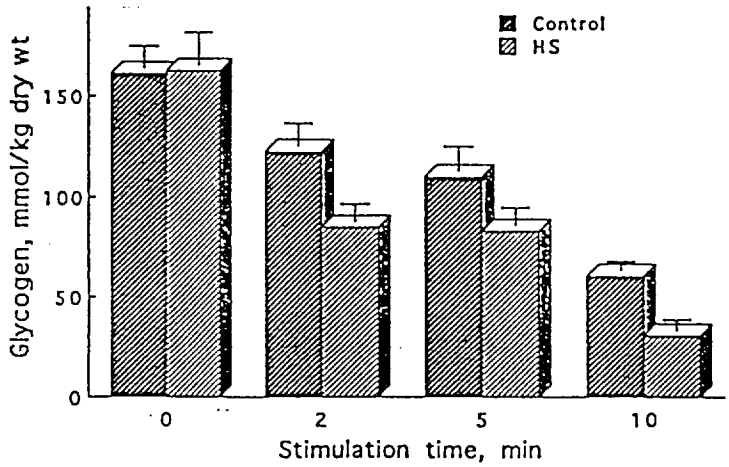


FIGURE 2.



The resting ATP and PC concentrations were not significantly different in fibers from the control and HS groups. With contractile activity, ATP showed little change (22.88 at rest to 19.08 mmole/kg dry wt after 10 min of stimulation), while PC declined to approximately the same amount in both groups (from a pre-stimulation value of 106 to 60 mmole/kg dry wt following 10 min of stimulation.

In a separate study, we evaluated the effect of low pH on the force generating capacity of single skinned soleus muscle fibers isolated from control and HS animals. Both Po and Eo declined with acidosis, but the Po/Eo ratio was depressed by 12% in control and by 25% following HS. These data indicate that the increased fatigability of the soleus following HS can in part be attributed to an enhanced susceptibility of the cross-bridge to the force inhibiting effects of the H^+ ion.

3. Exercise Countermeasures.

In this contract, we spent considerably effort evaluating the effects of two countermeasures- simple standing and resistive exercise in the form of ladder climbing. The standing group (HS-S) was let down from HS and allowed to stand for 10 min, 4 times per day. The resistive exercise group (HS-RE) climbed 20 cm up a vertical grid with a 500 g weight attached to their tails (1.5 times body wt).The rats performed 1 climb /min, 10 climbs/session, 4 times/day. Both HS-S and HS-RE showed only partial success in preventing soleus muscle atrophy. The control soleus muscle weights of 177 ± 8 mg were reduced to 107 ± 7 mg with HS, while the weights of the HS-S and HS-RE groups averaged 120 ± 6 and 126 ± 9, respectively (6). A partial protection against the HS-induced decline in fiber diameter and absolute force was also observed with both countermeasure programs, and importantly, the HS-RE program completely eliminated the HS mediated drop in force per cross-sectional area (Table 2).

TABLE 2. TYPE I FIBER DIAMETER AND FORCE

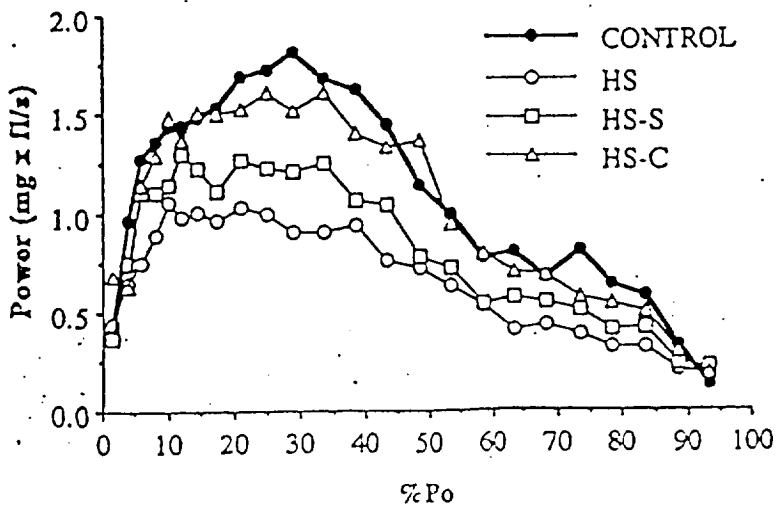
	Diameter (μm)	Po (mg)	P_o (kN/m^2)
Control	68 ± 1	50 ± 2	133 ± 3
HS	47 ± 2	20 ± 1	109 ± 4
HS-S	59 ± 1	34 ± 1	122 ± 2
HS-RE	60 ± 2	38 ± 2	130 ± 5

Neither countermeasure was successful in preventing the HS-induced increase in fiber V_o, but HS-RE did protect against the decline in peak power (PP) and the load at which peak power was obtained (%P_o at PP) in the single slow-twitch fibers of the soleus (Table 3 and Figure 4). In an attempt to find a better exercise countermeasure, we will in the future evaluate the effects of weight lifting in preventing the deleterious effects of HS on soleus muscle function.

TABLE 3. TYPE I FIBER MAXIMAL SHORTENING VELOCITY (Vo) AND PEAK POWER (PP)

	Vo (fl/s)	PP (mg.fl.s^{-1})	%P_o at PP
Control	1.24 ± .05	1.8 1± .1	29 ± 1
HS	1.59 ± .06	1.10 ± .1	16 ± 2
HS-S	1.40 ± .05	1.30 ± .1	19 ± 2
HS-RE	1.58 ± .11	1.70 ± .1	26 ± 3

FIGURE 3.



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