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RESPONSE OF RAT HINDLIMB MUSCLES TO 12 HOURS  
RECOVERY FROM TAIL-CAST SUSPENSION

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Hindlimbs of Female Sprague-Dawley rats were subjected to 6 days suspension followed by 12 h of weight bearing. The soleus (SOL), which atrophied with suspension, showed increased mass in recovery but without greater protein content. The extensor digitorum longus (EDL) and gastrocnemius showed reduced growth in suspension with no change of mass or protein in recovery. In contrast, the plantaris (PLN) recovered mass and protein after showing reduced growth in suspension. In the SOL during recovery, tyrosine decreased, and malate and aspartate, and the ratio of glutamine/glutamate increased. These changes were opposite to those occurring in suspension. The rise in glutamine/glutamate indicates greater ability of the muscle to produce glutamine. In the PLN during recovery, tyrosine decreased but no other significant changes occurred. The fall of tyrosine in SOL and PLN is probably indicative of improved protein balance. In the SOL, GAS and EDL during recovery, muscle alanine increased. These results show that the SOL is most responsive to resumption of loading presumably because of its marked atrophy during unloading.

Previous work from this laboratory has shown a number of biochemical changes which accompany atrophy or reduced muscle growth in hindlimb of tail-casted, suspended rats (1-5). These results clearly show that altered muscle growth was due to changes in protein turnover. Accordingly, the rise in soleus tyrosine following unloading reflects the more negative protein balance (5,6). Other major changes we found included slower synthesis of glutamine as indicated by lower ratios of glutamine/glutamate and reduced levels of aspartate which coincide with slower aspartate and ammonia metabolism in vitro (5,7). In conjunction with our study of SL-3 rats (6,7), which were subjected to 12 h of post-flight gravity, we carried out a study of the effects of 12 h weight bearing on metabolism of 6-day unloaded hindlimb muscles.

#### METHODS

Female Sprague-Dawley rats were subjected to 6 days of tail-cast, hindlimb suspension with or without a subsequent 12 h (overnight) period of normal weight bearing. Weight bearing controls were also studied but differences between weight

bearing and suspended are reported elsewhere (6,7). Muscles were excised rapidly, weighed and frozen in liquid nitrogen. Subsequently, pieces of muscle were sliced off and homogenized in 0.2 N perchloric acid (15-20 mg muscle/ml). After centrifugation, the supernatant solution was neutralized with KOH. All subsequent analyses were completed within 5 days. Certain metabolites (i.e., glutamine, glutamate, and malate) were assayed within the first 48 h by fluorometric or spectrophotometric methods (8-10). Other measurements included: tyrosine (11), aspartate (10), alanine (12) and protein (13).

#### RESULTS AND DISCUSSION

**Protein.** As a result of unloading (Table 1) the soleus lost protein while the gastrocnemius, plantaris and extensor digitorum longus muscles showed slower accumulation of protein than control muscles (+22%) and a smaller average increase than of the animals' body weights (+18%). Subjecting the animals to 12 h weight bearing after 6 days unloading did not change the difference in soleus protein loss but did produce a trend towards accumulation of protein more rapidly in the other hindlimb muscles.

**Tyrosine.** The loss of protein by the soleus muscle was associated with the accumulation of tyrosine which returned to control values during recovery. This 15% decrease of tyrosine in the soleus following unloading (Table 2) may reflect initial events of the muscle returning to a positive (growth) protein balance. The plantaris which showed the largest apparent accumulation of protein in recovery (+6% during 6 days of unloading to +18% during unloading + 12 h loading) also showed a significant decline in tyrosine (Table 2) even relative to the weight bearing muscle.

**Alanine.** Except for the plantaris, the other muscles showed increased alanine, especially the soleus. Since unloading does not decrease alanine, this change is not specifically one of recovery. Instead it may reflect some general change in whole body alanine metabolism. Further work is needed to understand this response.

**Glutamine Synthesis.** Both the soleus and gastrocnemius showed increased glutamine content during recovery. Glutamate content was unchanged. Only in the soleus was a significantly increased ratio of glutamine/glutamate measured. Based on our other studies of glutamine metabolism (3,5,7) these results reflect: a) a restored availability of intracellular ammonia for synthesis of glutamine and b) the elevated levels of glutamine synthetase. Increased ammonia availability probably results from renewed flux through the purine nucleotide cycle which generates ammonia via deamination of AMP. Glutamine synthetase activity is elevated as a result of glucocorticoid action.

**Aspartate and Malate.** Changes in aspartate coincide with the level of muscle activity (5,7) and are associated generally with parallel changes in malate. Coincident changes of malate and aspartate are not surprising since these compounds have the same carbon backbone structure. That the soleus muscle is beginning to recover with 12 h of loading is reflected in the rise of aspartate and malate levels (Table 2) to near normal. Aspartate levels are linked to muscle activity through the purine nucleotide cycle, in which aspartate provides the nitrogen for amination of IMP.

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Table 1. Protein and Tyrosine Content of Muscles With or Without Recovery

		Percent Difference from Weight Bearing			
		Soleus	Gastroc-nemius	Plantaris	EDL
Protein					
Unloaded	-19	+6	+6	+9	
Recovered	-22	+12	+18	+15	
Tyrosine					
Unloaded	+34	NS	NS	NS	
Recovered	NS	NS	-20	NS	

Table 2. Effect of 12 h Recovery on Tissue Metabolites

		Percent Difference from Unloaded			
		Soleus	Gastroc-nemius	Plantaris	EDL
Tyrosine	-15	NS	-21	NS	
Alanine	+120	+33	NS	+22	
Glutamine	+103	+24	NS	NS	
Gln/Glu	+91	NS	NS	NS	
Aspartate	+116	NS	NS	NS	
Malate	+74	NS	NS	NS	

**Concluding Remarks.** While measurement of the reaccumulation of muscle protein and mass provides absolute evidence of recovery, such changes in the atrophied soleus are not likely to be significant for several days. On the other hand, amino acids seem to return to their normal metabolic pattern in a matter of hours. Indeed, we have found normal glutamine/glutamate after only 4 h. Hence, amino acid measurements may provide sensitive indications of variations in muscle activity. This may be especially true in the case of aspartate and glutamine whose metabolism seems to be linked to flux through the purine nucleotide cycle as donor and acceptor, respectively, of nitrogen. With decreased muscle activity the use of ATP and hence the formation of AMP declines. The maintenance of a greater energy charge is likely responsible for slower deamination of AMP and hence the formation of nucleosides, the availability of ammonia for synthesis of glutamine and the utilization of aspartate for reamination.

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