Muscle Glycogenolysis and Exercise Following Rodent Hindlimb Suspension

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Abstract

The purpose of the study was to determine the effects of a 5 week hindlimb suspension period on rat muscle glycogenolytic responses at rest and during contractile activity induced by *in situ* muscle stimulation. After suspension, loss of muscle mass was greater in the soleus muscle (SOL, 58%) than in the plantaris muscle (PT, -17%). No significant change occurred at rest in the concentrations of creatine phosphate (PCr) or adenosine triphosphate (ATP) in control or suspended rats. No alteration was noted in PCr and ATP concentrations in SOL after stimulation of muscle contraction, but a large decrease (-30%) in PCr concentration was observed in PT in control and suspended muscles. Suspension did not affect glycogen concentration at rest in SOL or PT. In control rats, stimulation induced a decrease in glycogen concentration and an increased percentage of phosphorylase a only in the fast-twitch plantaris muscle while, in suspended rats, the same changes occurred in both muscles. These results are in accordance with the increased expression of fast-type myosin in the slow type I fibers in SOL (a load-bearing muscle) and the shift from oxidative to glycolytic metabolism previously demonstrated during simulated (hindlimb suspension) or real weightlessness.

Key words: muscle atrophy, stimulation, glycogenolysis, phosphocreatine, adenosine 5' triphosphate.

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Hindlimb unweighting has been commonly used in rodents as a ground-based model for simulating muscle atrophy [4,16] and bone demineralization [17] induced by weightlessness. Some metabolic consequences, such as an increase in glucose metabolism (glycogenesis, glucose oxidation, insulin stimulated 2-deoxy-D-glucose uptake), have been reported after short periods (6 or 7 days) of hindlimb suspension [6,8] in the soleus, a slow-twitch load-bearing muscle. This response is probably due to an increase in the glucose transporter (GLUT 4) protein content [7] and a 50% increase in concentration of insulin receptors due to a loss of muscle mass [8]. These results relative to carbohydrate metabolism contrast with those observed after denervation [14] or limb immobilization [1,15] where insulin resistance of glucose transport, glycogen synthesis and glycolysis was usually observed.

To delineate if the different adaptation of carbohydrate metabolism to unweighting could be due to the short duration of hindlimb suspension used in these studies [7,8], we evaluated the effects of a 5 week hindlimb suspension period on muscle glycogenolysis at rest and during contractile activity induced by *in situ* muscle stimulation.

Material and Methods

Animals

Twenty female pathogen free Wistar rats (200 g) from IFFA Credo (L'Arbresle, France) were used in the experiments. They were housed in a temperature controlled room (22 ± 2°C) with a light dark cycle (12:12h) and maintained on a diet of water and Purina laboratory chow. After a week of acclimatization to their new environment, half were suspended in individual cages using Morey's tail suspension model as previously described [4]. These animals were sacrificed immediately after the 5 week suspension period and the baseline control animals were sacrificed at the same time.

Electrical stimulation of muscle contraction

All the rats were initially anesthetized by inhalation of 2.5% halothane for 3-4 min. Anesthesia was maintained with 1.5% halothane (oxygen mixture introduced by face

mask) throughout the *in situ* muscle stimulation and muscular sampling procedures. The muscles chosen were the soleus, which consists mainly of slow-twitch fibers (85%) and the plantaris which is a fast-twitch muscle (30% fast twitch red, 50% fast twitch white fibers). The soleus and plantaris muscles in the right leg were exposed and dissected free of surrounding tissue, leaving its nerve and blood supply intact. The animals were then placed ventrally in a chamber maintained at 37°C. For muscle stimulation, the leg was fixed in a cradle with steel pins through the knee and ankle joints. The distal tendons of the two muscles (soleus and plantaris) were cut and secured to a a small steel hook with a silk suture for connection to a Grass model FTO3 force transducer. The sciatic nerve was severed in the gluteal region, the distal end of the nerve being drawn into a suction electrode. Supramaximal square-wave pulses of 0.5-ms duration were administered with a Grass S48 stimulator. The resting tension of the muscles was adjusted so that twitch tension was maximal. After a 5 min rest period to allow the muscles to recover from the adjustment to optimal length, the muscles were stimulated via the sciatic nerve for 15 sec with 100 ms long trains at 100 Hz delivered at a rate of 1/ s. At the end of the 15 sec of stimulation, the contracting soleus and plantaris muscles were clamp-frozen with aluminium tongs cooled in liquid N_2. The same muscles from the contralateral leg were also clamp frozen and used as resting control muscles. Muscles were kept frozen at 80°C until they were analyzed.

Muscle metabolites analyses

For glycogen and lactate determinations, a small muscle sample was homogenized in $HClO_4$ [13]. A portion of the $HClO_4$ homogenate was used for measurement of glycogen [11]. The remainder was centrifuged at 3000 g for 10 min at 4°C. The supernatant was removed, neutralized with KOH, and used for measurement of lactate [13]. Glycogen phosphorylase activity was measured as previously described [3].

For adenosine triphosphate (ATP) and creatine phosphate (PCr) analysis, muscle samples were weighed and immediately homogenized in ice-cooled 5% trichloroacetic acid, using a glass Potter-Elvehjem homogenizer. After centrifugation at 3000 g for 10 min, the supernatant was extracted four times with an equal volume of ether and then neutralized with 0.4 M triethanolamine hydrochloride-0.55 M (K_2CO_3). ATP and PCr were assayed by an enzymatic technique involving spectrophotometric determination of NADPH at 340 nm [12].

Statistical analysis

Statistical differences were determined by the Student t 's test, the level of significance was set at $P < 0.05$. Data are expressed as means ± SE.

Results

After five weeks of hindlimb suspension, the mass of the soleus and plantaris muscles were decreased by 58 and 17% respectively. In the soleus and plantaris muscles, the increase in lactate concentration occurring at rest after hindlimb suspension was at the limit of significance (Tables 1, 2). In control rats, stimulation of muscle contraction increased lactate concentration in both muscles.

Glycogen concentration was higher in the plantaris muscle compared to the soleus and was unaffected by five weeks of suspension (Tables 1, 2). Hindlimb suspended rats displayed a reduced glycogen concentration in the soleus and plantaris muscles after electrical stimulation, while control rats showed a significant decrease in glycogen concentration only in the plantaris muscle.

In control rats, stimulation of muscle contraction induced no change in the percentage of glycogen phosphorylase a in the soleus muscle (32.8 ± 4.1 at rest vs 38.5 ± 8.4) but increased the percentage in the plantaris muscle (41.1 ± 5.9 at rest vs 57.8 ± 5.2). In hindlimb suspended rats, phosphorylase activation was increased after stimulation either in the soleus muscle (34.2 ± 4.1 at rest vs 56.2 ± 4.3) or in the plantaris muscle (41.9 ± 7.3 vs 57.8 ± 4.2).

In control rats, concentrations of PCr and ATP at rest were significantly higher in plantaris than in soleus muscle (Tables 1, 2). At rest, no significant change occurred in the concentrations of PCr and ATP after 5 wk hindlimb suspension in either muscle. After stimulation of muscle

Table 1. Effects of hindlimb suspension (H 5wk) on soleus muscle metabolites at rest and after electrical stimulation.

	REST		STIMULATION	
	Control	H 5wk	Control	H 5wk
Glycogen	24.9 ± 1.5	26.6 ± 2.4	22.5 ± 1.9	16.4 ± 3.1 *
Lactate	2.5 ± 0.3	4.0 ± 0.7	3.6 ± 0.4 •	4.8 ± 0.4
ATP	5.2 ± 0.2	4.8 ± 0.3	5.0 ± 0.3	4.5 ± 0.4
PCr	12.9 ± 1.0	13.4 ± 0.8	10.6 ± 0.9	11.5 ± 1.1

Metabolites are expressed as µmol. g muscle-^{1}. Values are means ± SE.
• Significantly different after electrical stimulation in control rats.
* Significantly different after electrical stimulation in H rats.

TABLE 2. Effects of hindlimb suspension (H 5wk) on plantaris muscle metabolites at rest and after electrical stimulation.

	REST		STIMULATION	
	Control	H 5 wk	Control	H 5 wk
Glycogen	40.9 ± 2.4	34.4 ± 2.0	31.1 ± 3.4 •	28.1 ± 2.0 *
Lactate	2.1 ± 0.5	3.5 ± 0.7	4.9 ± 0.9 •	3.8 ± 0.7
ATP	6.3 ± 0.3	6.6 ± 0.3	6.4 ± 0.4	5.9 ± 0.5
PCr	20.6 ± 1.3	21.3 ± 1.4	15.0 ± 1.6 •	14.9 ± 1.1 *

Metabolites are expressed as µmol. g muscle^{-1}. Values are means ± SE.
• Significantly different after electrical stimulation in control rats.
* Significantly different after electrical stimulation in H rats.

contraction, PCr and ATP concentrations remained unchanged in the soleus muscle but a large decrease (-30%) in PCr concentration was observed in the plantaris muscle in control and suspended rats (Tables 1, 2).

Discussion

After 5 weeks of unweighting, a preferential atrophy of a slow-twitch ankle extensor muscle such as soleus was observed. These findings are in accord with those previously observed in the rat either after simulated microgravity induced by hindlimb suspension [4, 16] or true microgravity [5,9,10]. The present data showed no evidence of a direct influence of hindlimb suspension on soleus and plantaris glycogen concentrations at rest, in contrast to previous findings [8]. The greater concentration of muscle glycogen reported after 6 days of suspension [8] was observed in the soleus of growing rats (85-99g) while we studied adult rats in this experiment. On the other hand, the observed difference in the response of glycogen in the present experiment could be related to the longer duration of the suspension, 35 days instead of 6 or 7 days. Suspension induced a slight tendency to a higher lactate concentration in both muscles.

In control rats, *in situ* muscle stimulation induced a glycogen depletion only in the fast-twitch plantaris muscle, not in a slow-twitch muscle such as soleus. In suspended rats, a reduced glycogen concentration was observed either in the soleus or in the plantaris muscle. The mechanism responsible for the higher glycogenolysis during contractile activity in fast-twitch muscles, could relate to phosphorylase activity or to the difference in the concentration of inorganic phosphate (Pi) which is one of the substrates to phosphorylase. It appears from our preliminary findings that an activation of glycogen phosphorylase was shown in the fast twitch plantaris muscle of control rats. Moreover the same response was shown in the soleus and plantaris muscle of suspended rats. The resting concentration of ~ P (i.e. ATP and phosphocreatine) remained unchanged after hindlimb suspension in either muscle, but a significant breakdown of phosphocreatine with the liberation of Pi occurred in the plantaris muscle of control and suspended rats after stimulation.

It was previously shown that soleus muscle atrophy subsequent to prolonged suspension (5 weeks) was accompanied by an increased expression of fast-type myosin in the slow-type I fiber, which becomes a hybrid fiber [4]. A shift in the myosin heavy-chain (MHC) isoform distribution with a marked increase in the relative amount of type IIa and IIx MHC and a corresponding decrease in type I MHC was described [2]. The glycogen depletion which appears in the atrophied soleus muscle after stimulation of muscle contraction is in accordance with the increased expression of fast type myosin in the slow-type I fiber in SOL and the shift from oxidative to glycolytic metabolism, during simulated (hindlimb suspension, 4) or real weightlessness [5].

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