J. exp. Biol. ***176***, *135–144 (1993)*
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GLYCOGEN LOSS IN RAT MUSCLES DURING LOCOMOTION ON DIFFERENT INCLINES

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Accepted 1 October 1992

Summary

Running downhill causes structural damage in deep slow-twitch extensor muscles of the limbs. Both mechanical and metabolic hypotheses have been proposed to explain the damage. The purpose of this study was to use measurements of glycogen loss in the muscles and metabolic rates of rats running on the level and up and down 16° inclines at $26\,m\,min^{-1}$ to try to distinguish between these hypotheses. Glycogen loss in the soleus and medial head to the triceps brachii muscles during running on the three inclines was proportional to whole-animal oxygen consumption, indicating that there were no unusual metabolic demands on these muscles during the downhill exercise. The minimum area of these muscles showing glycogen loss was smaller during downhill than during uphill running. Average forces in the muscles are similar during locomotion on different inclines at the same speed, suggesting that stresses in the active motor units were greater during downhill running. Thus, the results are more consistent with a mechanical than with a metabolic etiology for the muscle injury resulting from downhill running.

Introduction

In this study, whole-body oxygen consumption ($\dot{V}_{O_2}$) and glycogen loss from foreleg and hindleg extensor muscles were measured in rats during treadmill locomotion at the same running speed at each of three different inclinations of the treadmill, i.e. level (0°) or up or down a 16° incline. Our original purpose was to gain insight into how patterns of fiber recruitment and proportions of active fibers in the muscles might differ between eccentric contractions, in which the muscles lengthen while active, and concentric contractions, which involve active shortening. Downhill locomotion biases the physiological extensor muscle contractions towards the eccentric type, whereas uphill locomotion biases the contractions towards the concentric type. The muscles perform both of these types of contraction in roughly equal proportions during each stance phase of the stride cycle in level locomotion. It was (and continues to be) of interest to know whether recruitment of the different motor unit types or volume of active muscle varied between the two modes of contraction. However, the glycogen loss data did not provide much insight into these questions, and the data have only been published in abstract form

Key words: metabolism, muscle, injury, exercise, oxygen consumption, rat.

(Armstrong and Taylor, 1980). More recently, one of us (Armstrong *et al.* 1983*b*) has used downhill locomotion in rats as a model for skeletal muscle strain injury, and the glycogen loss data from the earlier study provide useful information concerning whether this injury model has a metabolic or mechanical etiology.

Downhill locomotion causes injury to specific physiological extensor muscles in rodents (Armstrong *et al.* 1983*b*; Ogilvie *et al.* 1988; Duan *et al.* 1990) and humans (Fridén *et al.* 1981; Schwane *et al.* 1983). The injury consists of disruption of sarcomeres and other muscle structures followed sequentially by local inflammatory and regenerative processes (Armstrong *et al.* 1983*b*). It has been assumed that the injury to the muscles is due to accentuation of eccentric contractions in downhill compared to level locomotion, because eccentric contractions are known to damage skeletal muscles (Armstrong, 1984; Stauber, 1989; Armstrong *et al.* 1991). We (Armstrong *et al.* 1991) have favored the hypothesis that the initiating factors in the etiology of this muscle pathology are mechanical. For example, data from *in situ* (McCully and Faulkner, 1986) and *in vitro* (G. L. Warren, D. A. Hayes, D. A. Lowe and R. B. Armstrong, unpublished data) muscle injury models show that the injury is most closely related to peak forces produced during the eccentric contractions. Velocity of lengthening and initial length of the muscle are also related to the degree of injury. However, others have contended that the injury has a metabolic etiology (deVries, 1986; Lieber and Fridén, 1988). In favor of the metabolic hypothesis, the damage from eccentric contractions is histologically similar to that resulting from ischemia (e.g. Karpati *et al.* 1974; Makitie and Teravainen, 1977), and contraction of muscle in the presence of metabolic uncouplers can cause marked damage to fibers (e.g. Jackson *et al.* 1984).

We (Armstrong *et al.* 1983*b*) have used the downhill walking model in rats to induce injury in the deep slow-twitch extensor muscles in the foreleg, thigh and hindleg, i.e. triceps brachialis, medial head (TM), vastus intermedius (VI) and soleus (S), respectively. Downhill locomotion causes much greater damage to the muscles than level locomotion at the same treadmill speeds, even though downhill exercise elicits a lower whole-animal metabolic cost (Armstrong *et al.* 1983*a*). The slope of the line relating oxygen consumption ($\dot{V}_{O_2}$) to treadmill speed in rats is 23% less for locomotion down a 16˚ incline than that for level locomotion. However, it is possible that the metabolism of specific muscles (in particular, the deep slow extensors) or parts of muscles contributes disproportionately to the metabolic cost during downhill exercise. The purpose of this study was to use glycogen loss in the extensor muscles of the foreleg and hindleg of rats to estimate whether extraordinary metabolic demands are placed on the deep slow muscles during downhill compared with level or uphill locomotion. The data are not consistent with this hypothesis.

Materials and methods

Male Sprague-Dawley rats were used in these experiments. Rats (N=48) were obtained (Charles River Laboratory) when they weighed 200–225g and maintained in an environmentally controlled room at 23±2˚C. They were housed 2–4 to a cage and provided with food (commercial rat chow) and water *ad libitum*. All methods conformed

to the *Guiding Principles in the Care and Use of Animals* approved by the Council of the American Physiological Society.

One week after obtaining the animals they commenced a treadmill training program in which they ran at 16 m min^{-1} for 15min each on the level and up and down a 16° incline. The training program continued until at least 32 rats could run steadily at the front of the treadmill for 15min at each of the three inclines (0, +16 and −16°) at 26 m min^{-1}. This training period lasted 4 weeks.

Experimental protocol

Following the training program, oxygen consumption ($\dot{V}_{O_2}$) was measured while the rats ran on the treadmill on the level (0° incline) or up or down a 16° incline at 26 m min^{-1}. $\dot{V}_{O_2}$ was determined by an open-circuit method previously described in detail (Armstrong and Taylor, 1982; Armstrong *et al.* 1983*a*). These measurements were made over a period of 4 days. The regular training schedule was maintained in addition to the exercise during $\dot{V}_{O_2}$ determination. At the time of $\dot{V}_{O_2}$ measurement, the rat body masses ranged from 285 to 417g.

After determination of $\dot{V}_{O_2}$, and at least 2 days following the last training session, each rat ran for 5min on one of the inclines (0, +16 or −16°) at 26 m min^{-1}. The animal was not used if it did not run steadily at the front of the treadmill through the 5min of exercise. Immediately following exercise, the animal was anesthetized for 1–3min in ether, then decapitated. As rapidly as possible, the following muscles were removed for glycogen analysis: soleus (S), plantaris (P), gastrocnemius (G) and triceps brachii, medial (M), lateral (L) and long (Lg) heads. Upon removal, each muscle was halved. One of the halves was trimmed, mounted on a specimen holder and frozen in 2-methylbutane cooled in liquid N_2 for subsequent histochemical analysis. The second half was divided into two samples: one sample was weighed and placed in hot KOH for chemical glycogen analysis; the second was weighed, placed in a vial and desiccated in an oven at 60°C for at least two days for dry mass determination. For gastrocnemius muscle chemistry, the deep red portion of the lateral head (GR) and the superficial white portion of the medial head (GW) were sampled. Whole gastrocnemius muscles weighed 2245±103g (mean ± S.D.). GR samples weighed 95±23g and GW samples 82±17g. All muscles were in KOH or frozen within 8.0±0.9min of decapitation. We have previously found that very little change in glycogen concentration occurs in muscle samples in this time (Armstrong and Peterson, 1981).

Muscle analyses

Glycogen concentrations of the muscle samples were chemically measured with the anthrone procedure (Seifter *et al.* 1950). Using the wet mass/dry mass ratios, glycogen concentrations were expressed in mmolglycosylunitskg^{-1} drymass.

Serial sections of the muscle samples frozen for histochemistry were cut in a cryostat and assayed for reduced nicotinamide adenine dinucleotide–tetrazolium reductase (NADH-TR) activity (Novikoff *et al.* 1960), myofibrillar adenosine triphosphatase (ATPase) activity (Padykula and Herman, 1955) and glycogen with the periodic acid–Schiff (PAS) reagent (Pearse, 1961). From the sections treated for NADH-TR and

ATPase, the fibers were classed as fast-twitch oxidative glycolytic (FOG), fast-twitch glycolytic (FG) or slow-twitch oxidative (SO) using the system of Peter *et al.* (1972). Using the serial sections incubated with PAS, glycogen contents of at least 100 fibers of each fiber type in each cross section were subjectively estimated under the light microscope by assigning the fibers a staining intensity of 4, 3, 2 or 1 for those with dark, moderate, light or negative stains, respectively. Minimal percentages of fibers of each type and muscle cross sections showing glycogen loss were calculated as previously described (Armstrong *et al.* 1977; Sullivan and Armstrong, 1978; Armstrong and Taylor, 1982). The only data presented are those for estimated muscle cross-sectional areas showing glycogen loss.

Statistical analyses

A one-way analysis of variance and Duncan's new multiple range test were used to estimate differences among means for $\dot{V}_{O_2}$, muscle glycogen concentrations and muscle areas showing glycogen loss across groups. For linear regression analyses, a *t*-test was used to determine whether the slopes were different from zero. Means and slopes were considered different at $P \leq 0.05$.

Results

Steady-state $\dot{V}_{O_2}$ increased over pre-exercise levels during downhill, level and uphill locomotion (Fig. 1). $\dot{V}_{O_2}$ differed among the three exercise conditions; it was 18% higher during level than during downhill running, and 43% higher during uphill than during downhill locomotion.

Following 5min of downhill treadmill exercise at $26\,m\,min^{-1}$, there were no significant decreases in glycogen content in the extensor muscles in the foreleg or hindleg compared with unexercised control muscles, although all muscles showed absolute declines (Table 1). Following level running, S, GR and TM showed significant glycogen losses of 36%, 38% and 24%, respectively. After uphill running, all of the muscles with the exceptions of GW and TLg lost between 25 and 50% of their glycogen stores.

Table 1. *Muscle glycogen concentrations in mmolglucoseunitskg^{-1} drymass*

	Muscles						
Condition	S	P	GR	GW	TM	TL	TLg
Control (*N*=8)	167±8	164±15	167±14	187±16	168±20	181±14	183±14
Level (*N*=7)	107±12*	154±10	103±14*	166±12	120±12*	172±9	173±9
Downhill (*N*=8)	139±16	160±18	143±11	169±13	138±16	173±11	170±9
Uphill (*N*=6)	90±6*,†	111±15*,†	84±16*,†	159±18	99±16*,†	135±13*	152±10

Values are means ± S.E.M. for soleus (S), plantaris (P), and red (GR) and white (GW) portions of gastrocnemius and the medial (TM), lateral (TL) and long (TLg) heads of triceps brachii muscles.

*Values significantly lower than control ($P<0.05$).

†Values significantly different from that for downhill runners ($P<0.05$).

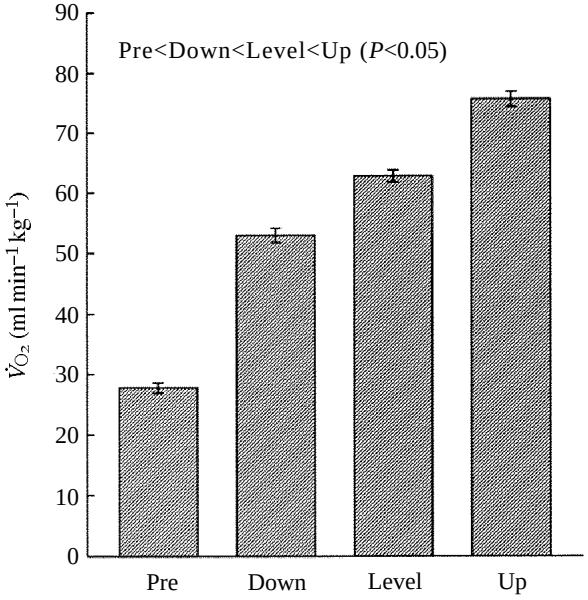

Fig. 1. Mean oxygen consumption ($\dot{V}_{O_2}$) ± S.E.M. for 10 rats standing on the treadmill before exercise (Pre) and during steady-state locomotion on the level treadmill (0°) and down and up a 16° incline at 26 m min^{-1}.

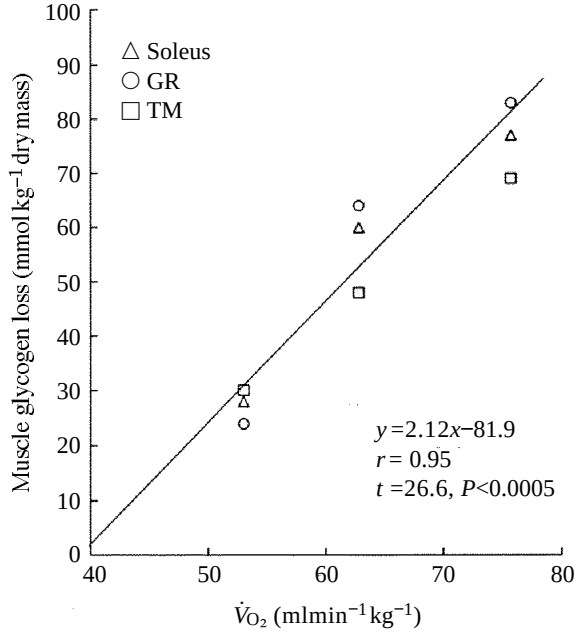

Fig. 2. Muscle glycogen loss, calculated as the difference between mean concentrations for control and exercised rats (Table 1), regressed on mean oxygen consumption for the animals during exercise on the respective treadmill inclines. Data are for soleus (S), red part of gastrocnemius (GR) and medial head of triceps brachii (TM) muscles.

Glycogen losses in the red muscles and muscle parts sampled in this study (S, TM and GR) were closely related to the whole-animal $\dot{V}_{O_2}$, as indicated by regression of the differences in glycogen concentration for each of these muscles between control and exercised conditions against $\dot{V}_{O_2}$ (Fig. 2). Thus, glycogen loss in red muscles during

Table 2. *Estimated muscle minimal percentage cross-sectional areas showing glycogen loss*

	Muscle or muscle group					
Condition	S	P	G	TM	TL	TLg
Level	42±9	28±7	29±6	57±8	35±11	33±9
Downhill	35±3	24±3	27±6	40±12	34±4	29±3
Uphill	47±9*	57±9†	33±6	78±5†	44±10	38±8†

Values are the mean percentage of minimal cross-sectional areas showing loss ± S.E.M. for soleus (S), plantaris (P) and gastrocnemius (G) muscles, and medial (TM), lateral (TL) and long (TLg) heads of triceps brachii muscles.

The number of rats in each group was 8.

*Mean value for uphill runners significantly higher than that for downhill runners (P<0.05).

†Mean value for uphill runners significantly higher than those for both level and downhill runners (P<0.05).

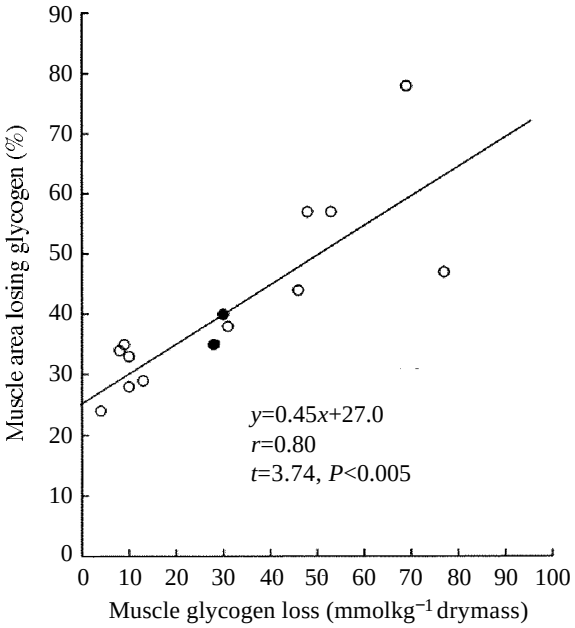

Fig. 3. Mean estimated muscle area losing glycogen from histochemistry (Table 2) regressed on mean muscle glycogen loss, calculated as the difference between mean concentrations for control and exercised rats (Table 1) during exercise on the respective inclines. Data for gastrocnemius muscle are not included because of sampling differences for chemistry and histochemistry. Filled circles are data for S and TM muscles.

downhill locomotion, both in muscles that show significant injury (i.e. S and TM) and in muscles that do not (i.e. GR), is closely related to whole-animal metabolism, suggesting that there is nothing metabolically unique about the downhill mode in the S and TM muscles.

Estimated minimal muscle areas showing glycogen loss are presented in Table 2. The muscle that had the largest minimal area showing loss after running at all three levels was TM; at least 40% of the muscle area showed loss after downhill running, and at least 78% after uphill running. In all muscles, the minimal area showing loss was smallest after downhill running and largest after uphill running. The primary reason for completing this analysis was to determine whether the glycogen loss measured in the whole-muscle samples after downhill running (Table 1) occurred in a disproportionately small (or large) cross-sectional area of the S and TM muscles. This would have indicated that a fraction of the motor units, or fibers, in the muscles were under more severe metabolic challenge during the downhill running exercise. The data for minimal areas showing glycogen loss do not indicate that there was anything extraordinary about the patterns of loss in the muscles; the areas showing loss generally appeared to be directionally similar to the whole-muscle glycogen loss data. To test this, the percentage muscle area losing glycogen was regressed on the chemically measured muscle glycogen loss data (Fig. 3). Data points for S and TM muscles are indicated with filled circles in the figure, and it is apparent that the relationship between area showing glycogen loss and whole-muscle glycogen loss are normal for these muscles.

Discussion

For animals the size of rats and larger the metabolic cost of running up an incline is greater than that for running down an incline at the same speed (e.g. Margaria, 1972; Taylor *et al.* 1972; Armstrong *et al.* 1983*a*). Also, downhill locomotion has a lower metabolic cost than level locomotion at the same speed (Armstrong *et al.* 1983*a*; Schwane *et al.* 1983). In running down an incline, the mass of the animal does work on the physiological extensor muscles, i.e. the muscles actively brake against the accelerating force of gravity. At the other extreme, during uphill locomotion, the extensor muscles actively shorten to lift the body mass, accelerating it against gravity. Although whole-body metabolism in most animals is relatively slow during downhill locomotion, the metabolic cost for individual muscles during downhill exercise could vary disproportionately with whole-body metabolism. This possibility has not been systematically explored.

The deep slow-twitch extensor muscles in the extensor groups, i.e. S and TM, sustain injury during downhill locomotion in rats (Armstrong *et al.* 1983*b*; Ogilvie *et al.* 1988; Duan *et al.* 1990); one reasonable explanation for this localized damage is that the eccentric contractions performed by the extensor muscles during downhill exercise in some way metabolically compromise the fibers, leading to initiation of the injury process. In support of this possibility, the later progression and histopathology of the injury in these muscles are similar to those resulting from ischemia (Karpati *et al.* 1974; Makitie and Teravainen, 1977). Thus, even though whole-body metabolism is slower during

downhill exercise, the possibility exists that high metabolic rates or ischemic conditions in specific motor units in the deep extensor muscles might be involved in the etiology of the injury that occurs.

Measurement of glycogen loss in the muscles during locomotion on different inclines should provide insight into this possibility. Increased rates of glycogen loss in the muscles would be expected either during periods of increased metabolism or during relative ischemia of the active fibers. Concerning the first of these possibilities, there is a well-described relationship between exercise intensity and glycogen loss rate in muscles (e.g. Saltin and Karlsson, 1972; Saltin and Gollnick, 1983). The higher the intensity of exercise and, hence, level of metabolism, the faster the loss of glycogen from the muscles. Glycogen loss in rat muscles increases with treadmill speed (Armstrong *et al.* 1974; Sullivan and Armstrong, 1978) and is greater during treadmill locomotion with a backpack load than during locomotion at the same speed without the load (Armstrong and Taylor, 1982). In contrast, reduced muscle activity in rats with partial curarization (Glenn *et al.* 1987) or denervation (Delp and Armstrong, 1988), respectively, decreases or prevents the loss of glycogen from the muscles during locomotion. Thus, the measurement of glycogen loss provides a semi-quantitative estimate of the relative level of metabolism in the muscle under defined conditions. An elevated glycogen loss also results from ischemia. Acute occlusion of arterial flow to one hindlimb of rats during treadmill locomotion increases the rate of glycogen loss in the muscles of that limb compared with the muscles in the normally perfused contralateral limb (Armstrong and Peterson, 1981). Thus, although measurement of glycogen loss during exercise does not provide a precise measure of the rate of metabolism in the muscles, it should permit a semi-quantitative estimate of whether metabolism is disturbed.

Data for glycogen loss from the muscles in this study provide no support for the hypothesis that abnormally fast metabolism or muscle ischemia is present in the deep slow-twitch muscles (S and TM) during downhill running. The loss of glycogen in S and TM muscles during level, downhill and uphill exercise was proportional to the whole-body $\dot{V}_{O_2}$ and similar to that in GR muscle, which does not show significant injury following downhill running (Armstrong *et al.* 1983*b*). If the glycogen loss that did occur in the muscles during downhill locomotion was restricted to a relatively small percentage of the fibers, it could be an indication that specific motor units or fibers in the muscles had abnormally high metabolic rates or ischemic conditions during the downhill exercise. However, estimates of the muscle cross-sectional areas showing glycogen loss did not indicate that this was the case. The relationship between estimates of muscle area losing glycogen and absolute glycogen loss (Fig. 3) for the muscles was reasonably good, and data for both muscles that sustain injury in the downhill model (S and TM) fell on the regression line. Thus, these findings provide no evidence for abnormal metabolic conditions in specific motor units or fibers in the muscles during the downhill protocol.

The findings of this study are consistent with the hypothesis that strain injury has a mechanical etiology. During locomotion at the same speed on different inclines, average forces exerted by the muscles are similar. However, the cross-sectional areas of active muscle in the deep slow extensor muscles were smaller during downhill than during uphill running. This was particularly evident in TM muscle, in which the area showing

glycogen loss during downhill running was about half that showing loss during uphill running. Thus, the average stress in the active motor units was higher during the downhill mode, because similar forces were produced by smaller cross-sectional areas of muscle. This interpretation must be made with care. First, the histochemical method of estimating glycogen loss is imprecise. Second, as discussed at length in previous papers (e.g. Armstrong *et al.* 1977; Sullivan and Armstrong, 1978), glycogen depletion patterns provide only a rough approximation of recruitment patterns. Finally, force production among synergistic muscles may be distributed differently during locomotion on different inclines. Nevertheless, the data are consistent with the hypothesis that the injury has a mechanical etiology.

In summary, we must be cautious in our use of glycogen loss to estimate metabolic conditions in the muscles during a preceding bout of exercise. However, the findings in this study provide no evidence that there is anything extraordinary about the metabolism of the S or TM muscles during downhill locomotion that distinguishes it from that during level or uphill exercise. Glycogen loss in the muscles during downhill running is reduced in proportion to the lower whole-body $\dot{V}_{O_2}$. Also, histochemical analysis for glycogen indicates there is nothing unusual about the proportions of fibers in the muscles losing glycogen, which could signal high specific metabolic rates in selected motor units or localized ischemia. However, smaller cross-sectional areas of the muscles lost glycogen during downhill than during uphill running. Because average forces in the muscles are similar during locomotion at the same speed on different inclines, this suggests that average stresses were higher during downhill running, supporting the hypothesis the injury has a mechanical etiology. Clearly the data do not disprove the hypothesis that the injury occurring in these muscles during downhill exercise has a metabolic etiology, but they provide no support for the metabolic hypothesis.

The authors wish to thank Mr Ron Phelps and Ms Judy Smith for their help with the technical aspects of this study and Ms Melinda Brewer for her help in preparing the manuscript. The study was supported by NIH Grant AR37098.

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