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INFLUENCE OF CIRCADIAN RHYTHMS ON RAT MUSCLE GLYCOGEN METABOLISM DURING AND AFTER EXERCISE

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SUMMARY

Marked circadian fluctuations in skeletal muscle glycogen concentrations have previously been reported. The purpose of the present study was to estimate the influence of these rhythms on muscle glycogen metabolism during and after high-intensity treadmill exercise. Male Sprague-Dawley rats ran five 1-min sprints at 75 m min^{-1} interspersed by 1–3 min rest intervals either at 08.00 h (morning) or at 20.00 h (night). All muscles sampled lost significant amounts of glycogen during exercise at both time periods. There were no differences in rates of loss between morning and night, even though glycogen levels in several muscles (high-oxidative muscles) were significantly higher before exercise in the morning. Following exercise, glycogen restoration in muscle samples primarily composed of fast-twitch fibres was more rapid in the morning than at night. There was no difference in glycogen restoration rates between the two time periods in the muscle primarily composed of slow-twitch fibres. Although liver glycogen was lower after exercise at night than in the morning, there were no differences in post-exercise blood glucose levels between the two time periods. In conclusion, circadian rhythms do not appear to influence rates of glycogen loss during high-speed running. However, since glycogen loss is the same at all times of day, one would predict that circadian changes in pre-exercise muscle glycogen concentrations would affect muscular endurance. Muscle glycogen restoration after exercise does appear to be affected by circadian rhythms, although interpretation of these data is complicated by possible changes in patterns of muscle fibre contraction at different times of the day. These circadian influences should be considered in the design of exercise studies using laboratory rodents.

INTRODUCTION

Significant circadian changes occur in the skeletal muscle glycogen concentrations of laboratory rats (Conlee, Rennie & Winder, 1976; Garwaite, Morgan & Meyer, 1979; Saubert & Armstrong, 1983). In fact, the nadir and acrophase of these daily rhythms in muscle glycogen levels may differ by as much as two-fold. The effects of

Key words: Fibre types, blood glucose, liver metabolism.

this large variation in muscle glycogen levels on the animal's metabolic response to locomotory exercise are unknown. Under certain conditions, exercise performance is directly related to pre-exercise levels of muscle glycogen (e.g., Bergström, Hermansen, Hultman & Saltin, 1967; Gollnick, Armstrong, Saubert *et al.* 1973). Also, Clark & Conlee (1979) recently demonstrated that circadian changes in liver glycogen limit the endurance of swimming rats. Recovery from heavy exercise would also presumably be dependent upon restoration of muscle glycogen. It therefore seems reasonable to expect that the marked changes in muscle glycogen concentration and in the hormonal or neural influences that regulate these changes might have important functional consequences on an animal's motor performance at different times during the circadian cycle.

In addition to furthering our understanding of the biology of rhythms, a knowledge of circadian effects on glycogen use and restoration in laboratory animals is of considerable practical importance. The data from studies using rats as experimental models are primarily useful when they can be generalized to other species, particularly man. Rats are nocturnal, but exercise studies using these animals are generally performed during the daylight hours. Thus, the animals might exhibit a different metabolic response to an exercise stimulus when forced to perform during the daytime. If the utilization and restoration of glycogen in response to exercise differs between day and night, then the diurnal effect should be considered in the design and interpretation of metabolic studies using exercising rats.

The present experiment was designed to test the hypothesis that circadian rhythms in laboratory rats influence muscle glycogen metabolism during and after high intensity treadmill exercise.

MATERIALS AND METHODS

Animals and training regimen

Male Sprague-Dawley rats with initial body weights of 150–200 g were purchased in units of 24 animals each. They were individually caged in a temperature-controlled room maintained at 24 ± 1 °C in which the day was artificially divided into 12 h of light (06.00–18.00 h) and 12 h of darkness (18.00–06.00 h). Food (commercial pellet chow) and water were provided *ad libitum*.

The rats were taught to run 60 s bouts at 75 m min^{-1} on a motor-driven treadmill for 2 weeks (5 days per week). During the first several days of the training period the animals performed 1–3, 30 s runs at 13·4 m min^{-1}. Both the speed and the number of runs were increased until the rats could complete 5 consecutive 1 min runs at 75 m min^{-1} with 1–3 min of rest between runs. All 24 animals in each unit were trained during the same time period (usually 10.00–13.00 h) each day during the familiarization period.

Experimental protocol

Twenty-four hours after the last training period 20 animals were selected from each unit for their running ability. These rats were divided into four groups. One group (day, exercise: DE) was exercised 2 h after the initiation of the light cycle (08.00 h).

This time was chosen because most of the rat experiments in our laboratory are carried out in the morning. A second group of rats (night, exercise: NE) was exercised 12 h later, or 2 h after the beginning of the dark cycle (20.00 h). The other two groups of animals served as non-exercised controls (day, control: DC; and night, control: NC, respectively). The animals in the exercise groups (DE and NE) were first warmed up for 1 min by running at 26·8 m min^{-1}. They then performed 5, 1 min runs at 75 m min^{-1} with 1–3 min of rest between runs. The rats were bled to death after 1 min of ether anaesthesia at either 0, 0·5, 1, 2 or 4 h following the last high-speed run. Non-exercised control animals (DC and NC) were killed at the same points in time. The weights of the animals when killed averaged 348±65 g (S.D.).

Tissue analysis

Blood samples were taken from the abdominal aorta of the rats as they were being killed and were subsequently analysed for glucose using the Glucostat method (Worthington Biochemical). Samples (10–20 mg) of the red and white portions of gastrocnemius muscle (RG and WG, respectively), soleus muscle (S), and liver (L) were then rapidly excised. The RG and WG samples were taken from the deep portion of the lateral head and the peripheral portion of the medial head of the gastrocnemius muscle, respectively. A cross-section from the middle ¼ of S was used. The fibre type compositions of these muscle samples are included in Table 1. The L sample was removed from the medial rim of the left lobe of the liver.

The muscle and liver samples were frozen in liquid N_2 within 3 min following death. Insignificant amounts of glycogen are lost from the tissues in this time (Armstrong & Peterson, 1981). They were subsequently prepared for glycogen analysis with the technique described by Sembrowich, Knudson & Gollnick (1977) and analysed for glycogen concentration using the fluorometric procedures of Lowry & Passonneau (1972).

Statistical analysis

Statistical differences among mean glycogen concentrations within groups across times, and among groups within times, were ascertained using a factorial analysis of variance and Scheffe's multiple comparison procedure employing Snedecor's F-Distribution (Snedecor & Cochran, 1967). Differences between day and night rates of glycogen utilization and restoration were tested with Student's t-test by comparing slopes calculated from linear regression. Two mean values or slopes were considered different if they attained at least the 0·05 level of significance.

Table 1. *Fibre type composition of the muscle samples*

Muscle sample	Fibre type (%)		
	SO	FOG	FG
Soleus	77 ± 9	23 ± 9	0 ± 0
Red gastrocnemius	34 ± 13	60 ± 6	6 ± 10
White gastrocnemius	0 ± 0	10 ± 1	90 ± 1

Values are means ± S.D. for three rats. Data are taken from Laughlin & Armstrong (1982).
SO, slow-twitch, oxidative; FOG, fast-twitch, oxidative glycolytic; FG, fast-twitch, glycolytic.

RESULTS

Changes in glycogen concentrations with exercise and during the post-exercise period for S, RG, WG and L are presented in Figs 1, 2, 3 and 4, respectively. Blood glucose data are contained in Table 2.

Diurnal effects in non-exercised rats

Comparisons among sampling times within DC and NC groups

No differences were observed in the average glycogen concentrations in S, RG, WG or L among the four sampling times in either the DC or NC groups. Similarly, the mean values for blood glucose concentration were the same among the DC time periods. However, mean blood glucose values for the NC animals in the 0 and 0·5 h groups were lower than those for the 4 h group.

Thus, significant circadian effects on muscle or liver glycogen levels were not observed over the 4 h sampling periods either in the morning or evening.

Comparisons between DC and NC groups

Glycogen concentrations in S and RG were significantly lower at 20.00 h (NC) than at 08.00 h (DC) (0 h in Figs 1 and 2). Thus, there was a difference in pre-exercise glycogen levels in these muscles between the two time periods. However, at the 30 min

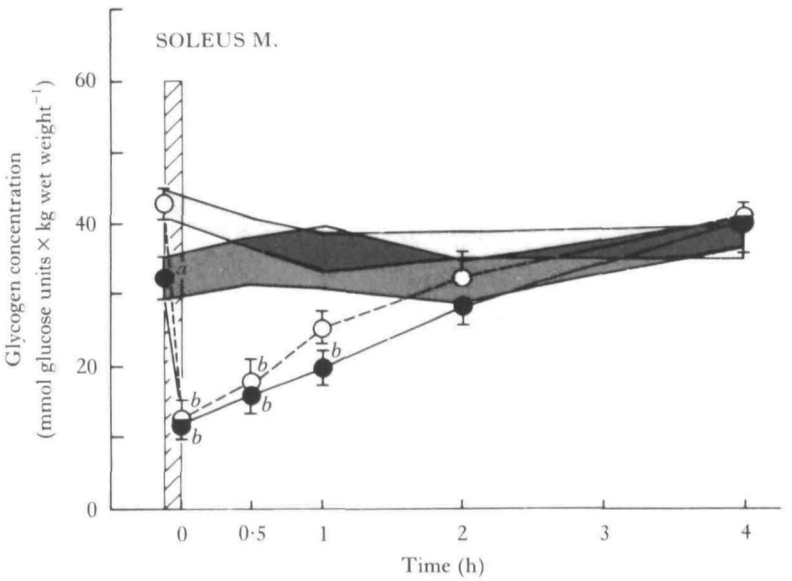

Fig. 1. Glycogen concentrations in soleus muscle of day control (light shading, $\bar{x} \pm$ S.E.), night control (dark shading, $\bar{x} \pm$ S.E.), day exercise (open circles, $\bar{x} \pm$ S.E.), and night exercise (closed circles, $\bar{x} \pm$ S.E.) groups before and after exercise over time. The exercise period is indicated by the cross-hatched bar. *a*, Mean value for night significantly lower than the respective daytime value ($P < 0{\cdot}05$). *b*, Mean exercise value significantly lower than the respective control value ($P < 0{\cdot}05$). For each mean, $N = 4$–9.

post-exercise sampling time and for the next 3·5 h there were no differences between DC and NC for any variable.

Glycogen loss during exercise

All three muscles, S, RG and WG, demonstrated significant losses of glycogen during the 5 min of treadmill running at both time periods. Rates of glycogen loss during the 5 min of exercise may be calculated if: (a) the rate was constant through the five exercise bouts; and (b) glycogen restoration was minimal during the rest periods between exercise. If these assumptions are made, RG in the NE group showed the highest rate of loss, and WG in the DE and NE groups the lowest rate of loss (6·9 and 4·2 mmol glucose kg^{-1} min^{-1}, respectively). There were no significant differences in the rates of loss between DE and NE for any of the three muscles studied, indicating there were no significant circadian effects on glycogen loss in the muscles at the time periods studied.

Liver glycogen levels in the DE and NE groups were not significantly lowered below their respective controls immediately after exercise (0 h), nor were the calculated rates of loss for DE and NE different during exercise. However, the immediate post-exercise (0 h) glycogen concentration in NE was lower than that for DE. Exercise had no significant effect on blood glucose concentrations either during the day or night.

Glycogen restoration following exercise

Glycogen restoration rates in RG during the first 30 min after exercise were significantly different between DE and NE (0·9 and 0·5 mmol glucose kg^{-1} min^{-1},

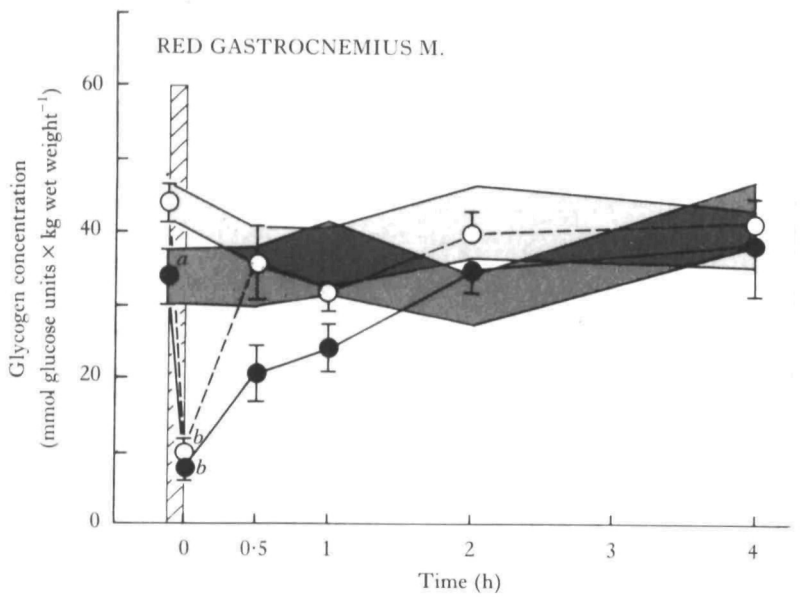

Fig. 2. Glycogen concentrations in red gastrocnemius muscles over time. The symbols are described in the legend for Fig. 1. For each mean, $N = 4$–9.

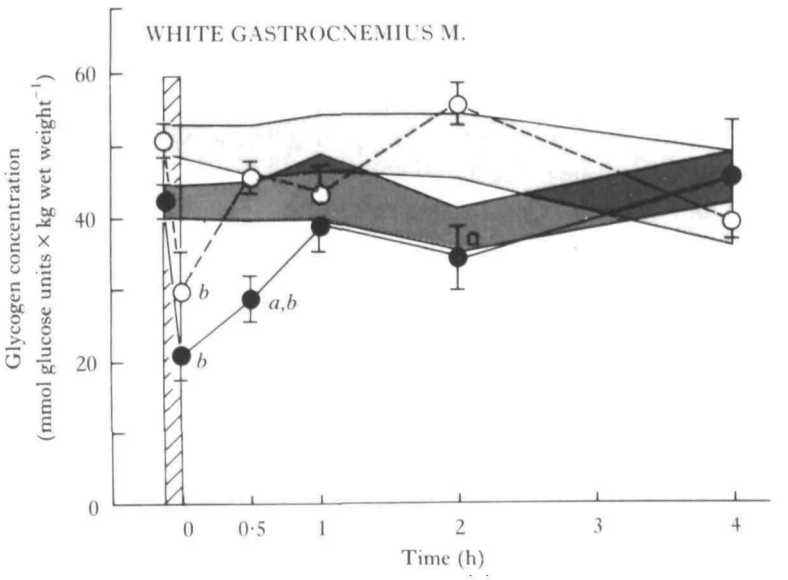

Fig. 3. Glycogen concentrations in white gastrocnemius muscles over time. The symbols are described in the legend for Fig. 1. For each mean, $N = 4$–9.

respectively) (Fig. 2). In both DE and NE groups the glycogen concentrations 30 min after exercise in RG were not different from their respective control groups. No further significant changes occurred in either DE or NE over the remainder of the experimental period in this muscle. Although the DE glycogen restoration rate in WG was 67 % higher than in NE (0·5 and 0·3 mmol glucose kg^{-1} min^{-1}, respectively), the difference was not significant (Fig. 3). However, 30 min after exercise the glycogen concentration in DE was not different from control, whereas that for NE was still significantly lower than that for either NC or DE. One hour after exercise, glycogen levels in WG from both DE and NE were not different from control.

The slowest glycogen restoration rates were observed in S (Fig. 1) in the DE and NE groups (0·2 mmol glucose kg^{-1} min^{-1} at both times). Thirty minutes after exercise, S glycogen levels in both groups were still significantly lower than their respective controls. One hour after exercise, DE glycogen concentration in S was not different from control, whereas for NE it was still low.

Liver glycogen concentrations in the DE group were not different from control values throughout the experimental period (Fig. 4). The only time period in which NE had lower L glycogen levels than NC was 2 h after exercise, although at several time periods NE was lower than DE. The only significant difference between blood glucose levels at the various times after exercise was at 1 h when NE was higher than NC (Table 2).

DISCUSSION

This study was designed to evaluate the influence of circadian rhythms on rat muscle glycogen metabolism during and after high-speed treadmill running. The

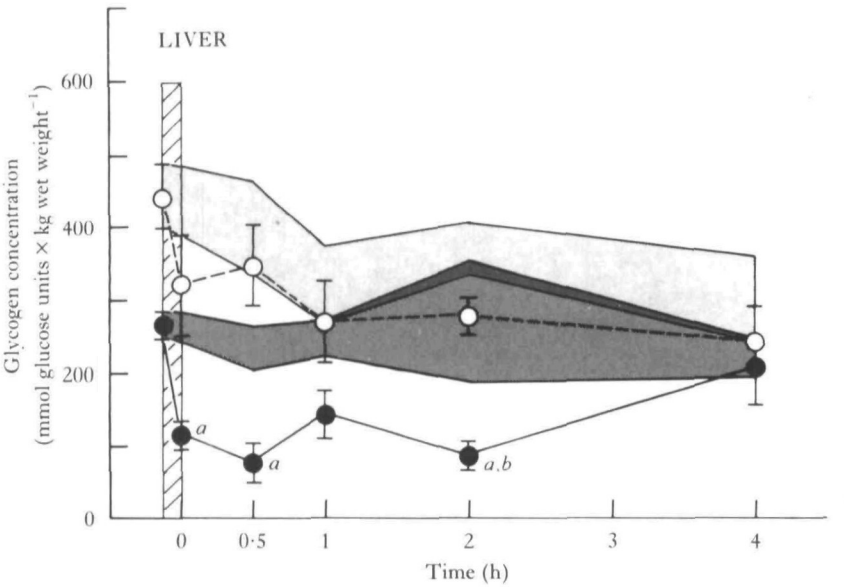

Fig. 4. Glycogen concentrations in liver over time. The symbols are described in the legend for Fig. 1. For each mean, $N = 4$–9.

findings indicate that at the two time periods studied the rates of muscle glycogen usage were similar during exercise, but that differences in glycogen restoration in the muscles after exercise existed between day and night.

Muscle glycogen levels before exercise were generally lower at night than they were during the morning. However, the rates of glycogen usage during the high speed running were the same at the two time periods in each of the three muscles. Thus, the rates of muscle glycogen usage apparently were not affected by the initial concentrations in the muscles. At lower relative exercise intensities the rate of glycogen loss is directly related to the initial glycogen concentration (Pernow & Saltin, 1971; Gollnick *et al.* 1972). Thus, the relationship between initial glycogen content in the muscles and the rate of glycogenolysis during exercise varies with exercise intensity. Interestingly, Clark & Conlee (1979) observed a greater rate of loss in white vastus lateralis muscle when rats swam at night than during swimming in the morning, even though the pre-exercise glycogen concentration in the muscle was lower at night. The authors suggested that the increased loss in the white muscle at night resulted from depletion of glycogen in, and resulting fatigue of, high-oxidative fibres, requiring increased recruitment of white fibres. However, the rates of loss of glycogen in red vastus lateralis and soleus muscles were the same in the morning and at night, and even at night, when the muscles started at lower initial glycogen levels, they did not completely deplete their glycogen stores. The differences between the results of Clark & Conlee (1979) and those of the present study emphasize the importance of the influence of the type and intensity of exercise on glycogen metabolism in the muscles.

The importance of glycogen to muscle metabolism during high intensity exercise

Table 2. *Blood glucose concentrations for day (D) and night (N) groups of rats*

Group	Time post-exercise (h)									
	0		0·5		1		2		4	
	D	N	D	N	D	N	D	N	D	N
Control	5·07 ± 0·12 (3)	5·57 ± 0·46 (2)	5·73 ± 0·20 (3)	6·05 ± 0·01 (2)	6·01 ± 0·39 (3)	5·78 ± 0·41 (2)	5·87 ± 0·23 (3)	6·26 ± 0·25 (2)	5·90 ± 0·35 (2)	7·43 ± 0·18 (2)
Exercise	5·08 ± 0·54 (3)	5·70 ± 0·38 (5)	5·83 ± 0·36 (3)	6·22 ± 0·63 (6)	6·60 ± 0·20 (6)	7·66* ± 0·26 (5)	6·42 ± 0·42 (3)	7·38 ± 0·47 (4)	5·87 ± 1·01 (5)	7·69 ± 0·57 (3)

Values are means ± S.E. Blood glucose concentrations are given in mM. Number of animals (*N*) is included in parenthesis.
*Day exercise different from day control ($P < 0·05$).

has been amply demonstrated (e.g., Saltin & Karlsson, 1971; Gollnick, Armstrong, Sembrowich *et al.* 1973). Depletion of muscle glycogen stores, in fact, appears to be responsible for muscular fatigue under certain circumstances (Bergström *et al.* 1967; Hermansen, Hultman & Saltin, 1967; Gollnick, Armstrong, Saubert *et al.* 1973). Thus, the large circadian change in muscle glycogen concentrations that has been observed (Conlee *et al.* 1976; Garwaite *et al.* 1979; Saubert & Armstrong, 1983) might be expected to affect an animal's ability to perform high intensity exercise. Presumably this could have important consequences for an animal that is escaping from predators or capturing prey during a high-speed chase. It is not known if wild animals experience circadian changes in muscle glycogen of the magnitude of those observed in laboratory rodents, but the potential importance of such changes to the animal's behaviour is obvious.

The major circadian effect on glycogen metabolism observed in this study was on glycogen restoration rates after exercise. The rates of muscle glycogen restoration following exercise were higher in the fast-twitch muscles during the morning than in the evening. In S muscle, the rates of restoration were the same at the two time periods. One variable that could affect glycogen resynthesis rates is the blood glucose concentration. However, there were no differences in blood glucose between either control or exercise day and night groups at any time period, so differences in availability of glucose for glycogen synthesis were not responsible for the differences in glycogen restoration rates in fast-twitch muscles between the day and night groups. Liver glycogen concentrations were significantly lower after exercise at night than during the daytime at several sampling times, but the levels were adequate to maintain blood glucose concentrations.

Failure to observe a difference in glycogen restoration rates between morning and evening in S may reflect the specific contractile function of the muscle. In cats, S is maximally active during quiet standing to provide postural support (Smith, Edgerton, Betts & Collatos, 1977; Walmsley, Hodgson & Burke, 1978). Presumably the muscle functions similarly in the rat. Muscle contraction favours phosphorylase activation and glycogenolysis, while the activity of glycogen synthetase is reciprocally diminished (Soderling *et al.* 1970; Staneloni & Piras, 1969). Thus, in a postural muscle like S that is continuously active glycogen restoration might be expected to be retarded. Terjung, Baldwin, Winder & Holloszy (1974) previously reported that the glycogen resynthesis rate in S after exhaustive swimming was about half that in red vastus lateralis muscle, which is primarily composed of fast-twitch muscle fibres.

Red gastrocnemius muscle is composed primarily of fast-twitch, oxidative-glycolytic muscle fibres (Table 1). These fibres are probably not active during postural maintenance, but are recruited at low levels of force production such as during walking (Armstrong *et al.* 1977). The rate of glycogen restoration found during recovery from exercise at night in this muscle was significantly lower than that observed during the day. This suggests that a circadian effect on glycogen resynthesis was present in this muscle. However, the contractile activity of red gastrocnemius muscle at night, when the animal is most active, might be expected to slow the rate of glycogen restoration through the reciprocity of the phosphorylase-glycogen synthetase system. It is interesting to note that the lower rate of restoration at night occurred even though the animals feed at night and hormonal conditions would favour glycogen storage

(Conlee *et al.* 1976). This emphasizes the importance of considering the contractile activity of the muscles in the interpretation of the data.

With this in mind, white gastrocnemius muscle may provide the best information on circadian influences on muscle. It is composed primarily of fast-twitch, glycolytic fibres (Table 1). These muscle fibres have been shown to be recruited only if the exercise is of a high intensity, or when the animal's oxidative fibres become depleted of glycogen (Gollnick, Armstrong, Saubert *et al.* 1973; Armstrong *et al.* 1974, 1977; Sullivan & Armstrong, 1978). Therefore, the fibres in this muscle are probably not active either during postural maintenance or during routine cage activity. Muscle fibre contractile activity would therefore not be expected to obscure any circadian changes in the glycogen metabolism of white gastrocnemius muscle while the animals are in their cages. The observation that glycogen restoration rates at night are about 40 % lower than those during the day in white gastrocnemius muscle would suggest that there is a true circadian effect on muscle glycogen restoration. However, this point remains speculative because the restoration rates at the two times were not significantly different ($0{\cdot}1 > P > 0{\cdot}05$). It is also of interest to note that the higher rate of glycogen resynthesis in white gastrocnemius muscle occurred in the daytime when the initial post-exercise concentration was higher than that at night. This is somewhat surprising in view of the findings of Danforth (1965), who demonstrated that glycogen synthetase I activity in muscle is inversely related to glycogen concentration in the muscle. He concluded that glycogen controls its own synthesis, so that when levels are high the enzyme is less active. Our data serve as an example of how control mechanisms that operate *in vitro* may be overridden in the intact animal.

The data from this study demonstrate the importance of considering circadian rhythms in designing experiments on muscle metabolism in laboratory rodents, supporting the conclusions of Conlee & Clark (1979). They reported that differences in liver glycogen content resulting from circadian changes affected the endurance time in swimming rats. Rats that swam in the evening (19.00 h) had initial liver glycogen concentrations 46 % lower than those that swam in the morning (07.00 h), and showed a 39 % reduction in swim time. The authors concluded that fatigue in the swimming animals at both time periods resulted from hypoglycaemia secondary to liver glycogen depletion. All muscle samples contained glycogen at the time the rats stopped swimming, so fatigue in this form of low-intensity, long-term exercise did not result from complete depletion of muscle glycogen stores. Presumably, the hypoglycaemia had central effects that limited the animals' performances.

The physiological mechanisms that regulate daily rhythms in skeletal muscle glycogen metabolism are not known. The fact that the various muscle fibre types and liver demonstrate the same general patterns in their circadian changes in glycogen levels (Conlee *et al.* 1976; Saubert & Armstrong, 1983) indicates that a central neural or hormonal regulation is responsible. However, this central mechanism has not been identified. Changes in food intake (Garwaite *et al.* 1979), insulin (Conlee *et al.* 1976; Saubert & Armstrong, 1983), or catecholamines (Saubert & Armstrong, 1983) do not appear to account for the circadian rhythms in glycogen levels in skeletal muscle. Rhythms in glucocorticoid levels (Dunn, Scheving & Millett, 1972) seem to have the proper phase relationships to affect the observed changes in muscle glycogen concentrations, although a possible causal relationship remains to be demonstrated.

In conclusion, at the two time periods studied (08.00 and 20.00 h), there were no differences in rates of glycogen loss during high-speed running in any of the muscles studied. However, muscles primarily composed of fast-twitch fibres did show differences in rates of glycogen restoration between the two time periods, indicating that circadian rhythms may influence muscle glycogen metabolism after exercise. The data do not shed light on the mechanisms that could control rhythms in muscle glycogen regulation.

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